A MATHEMATICAL MODEL OF BIOFILM GROWTH AND DECAY

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A MATHEMATICAL MODEL OF BIOFILM GROWTH AND DECAY

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Thesis

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ABSTRACT

A biofilm is a community of microorganisms embedded in a matrix of proteins, nucleic acids, and polysaccharides. It has been noted that bacteria growing in biofilm conditions are much more resistant to antimicrobials than those bacteria growing in non-biofilm conditions. This paper describes a mathematical model of biofilm growth and decay. The model includes a set of reaction-diffusion equations used to describe the movement of soluble components of the biofilm such as nutrients and antimicrobial agents, and a set of population equations for the description of the particulate components of the biofilm such as forms of bacteria and the component providing structure to the biofilm known as extracellular polymeric substance (EPS). The aim of this paper is to describe a two-dimensional model and the subsequent assumptions made to reduce and solve it as a one-dimensional model. The one-dimensional model that results will be solved using ordinary differential equation solution techniques. From this simplified model, it has been shown that topical treatment with antimicrobial as well as nanosphere delivery for antimicrobial are effective treatment options. Moreover, the modelled mechanism of resistance for bacteria growing in biofilms, 'persister' bacteria, was observed. Living bacteria growing with high initial persister populations showed less growth than those living bacteria growing with low initial

persister populations. The parametric study between the terms k_f and k_R (rate of formation of persisters and rate of reversion to living bacteria, respectively) shows that change in these values affects bacterial population sizes, but does not seriously affect the minimum inhibitory concentration (MIC) of the antimicrobial. In conclusion, a summary for calculating proper dosage levels of antimicrobial with nanosphere delivery is performed showing the application of the model to drug-testing.

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CHAPTER I

INTRODUCTION

A bacterial biofilm is a community of bacteria growing in a liquid matrix of proteins, nucleic acids, and polysaccharides. Biofilms are of interest not only due to their ubiquity, but also since bacteria growing in biofilm conditions have higher resistance to antimicrobials than bacteria growing in non-biofilm conditions [1].

Most notable for this paper, biofilms are pervasive in the medical field. Dental bacterial biofilms, familiarly known as plaque, are responsible for periodontal disease and dental caries which have devastating effects on the body's chief tool for the initial phase of nutrient breakdown [2, 3]. In addition to their effect on the human mouth, biofilms are also problematic in catheters used in hospitals. Biofilms attach and grow on both Foley catheters used for draining the bladder and central venous catheters used in patients unable to eat by mouth thus requiring parenteral nutrition [2, 4, 5]. These catheter biofilms lead to nosocomial (hospital-acquired) infections, further complicating a critical patient's health status. Unfortunately, biofilms are also commonplace in the bronchial passageways leading to the lungs in a large group of immunocompromised patients, particularly, those patients suffering with Cystic Fibrosis (CF) [6]. The disease process of CF leads to biofilm formation in the bronchi which elicits an exaggerated inflammatory response from the host. This exagger-

ated response leads to mucous formation which in turn encourages biofilm formation, putting CF patients in a cycle of inflammatory responses that is difficult for them to escape [7]. This paper seeks to describe a relevant mathematical model for the growth and decay of biofilms in the bronchial passages leading to the lungs.

Many models of biofilms already exist in the literature today. Of these, there are three main classes. The first class models the components of the biofilm as a continuum, so that all bacteria in the biofilm are treated as one entity that is continuous as seen in [8, 9, 10, 11, 12, 13]. The second class models the individual bacteria as separate entities and falls into the category of a discrete model as seen in [14, 15]. In these models, the domain of the biofilm is partitioned into compartments housing bacteria; the models then employ an algorithm that determines the easiest path for the bacterial cells to travel to create a biofilm. These algorithms assume that adjacent compartments that are not filled to capacity will be easier for the bacteria to occupy, and dictates that bacteria seek adjacent compartments with higher available resources. In [15], mass balances are also exploited to explain the transport of bacterial cells through the biofilm domain thus utilizing fundamental principles. These models give a realistic view, as bacteria are discrete entities. In addition, these models enable for a heterogeneous view of biofilm composition, demonstrate the cooperation and competition exhibited by bacteria in biofilm growth, and provide valuable insight into the non-uniformity in density of some biofilms. The third class is a hybrid of the two aforementioned classes and combines both a continuous model for some components of the biofilm, and a discrete model for others in works like

[16, 17]. In this work, a model of the first class is presented, where each of the biofilm constituents is treated as a continuous quantity.

There are varying theories as to why bacteria growing in biofilm conditions display higher resistance to antimicrobials than their planktonic counterpart. Five theories of interest are: inability for antimicrobial to penetrate living bacteria, ability for biofilms to neutralize antimicrobials by reaction, substrate limitation causing inactive bacterial cells that are non-responsive to antimicrobials, the ability for cells to adapt to surrounding bacteria through a process known as quorum-sensing, and the ability for a living bacterial cell to transform freely into a non-respiring cell termed a 'persister' that is not susceptible to antimicrobials [2, 9, 10, 11, 17, 18, 19, 20, 21, 22]. The theory that antimicrobials may be unable to penetrate bacteria does explain bacterial resistance to some antimicrobials, but does not explain why bacteria living in biofilm conditions would be more resistant than those not growing within in a biofilm and has been been discounted with some experiments [20, 23]. The reaction theory explains that the biofilm is able to neutralize antimicrobials at surface levels of the biofilm so that at deeper levels of the biofilm an antimicrobial is rendered ineffective. Some claim that biofilms are dense enough to not allow antimicrobials past their surface level [24]. These theories' drawback is that it only could explain a biofilm's resistance if it were relatively thick. Moreover, many antimicrobials have been shown to successfully penetrate into biofilms [3]. Nonetheless these theories may be valid when considered in conjunction with one of the other theories [17]. The third theory, claiming that bacteria inhabiting an area of low concentration of substrate within

the biofilm are inactive to antimicrobial, is promising. However, it is questioned why when surrounding bacteria are killed (those thought to be in areas of high concentration of substrate), that substrate does not transport to the areas of previous low concentration making dormant cells again viable and thus responsive to antimicrobial [17]. Quorum-sensing is thought to be similar to the process involved in an efflux-pump, and is a viable explanation for increased resistance [2, 9, 12, 17, 18, 19]. The last mechanism of resistance mentioned, the presence of persister cells, is one incorporated in this model. It is thought that living cells can become a phenotypic variant known as persister where they are completely unresponsive to antimicrobial. The belief is that these persisters are able to transform back into living cells, which are able to continue to grow after antimicrobials are no longer present [2, 11, 17, 21, 22].

The literature describes many components of a biofilm. This paper seeks to model as many of these components as possible into one cohesive model. The biofilms modelled in this paper comprised four major particulate components: extracellular polymeric substance (EPS), living bacteria, dead or inert bacteria, and persister bacteria. EPS is the substance that is responsible for giving the biofilm its structure and is produced by living bacteria [2]. Cogan focuses solely on the modelling of EPS in [8] and others include EPS in more general models as in [1, 11, 13, 14, 16, 19]. In the model, living bacteria are the only particulate capable of producing EPS; capable of dividing (producing more living bacteria); capable of dying; capable of transforming into persister; and the only bacteria population that is responsive to antimicrobial and nutrient. The production of EPS is dependent on the number of

bacteria and our model of EPS production shares similarities with [1, 8, 13]. While this model proposes modelling of the biofilm resistance via transformation from living bacteria to persisters similar to [11, 17], others propose that persisters act as separate entities from living bacteria with no means for reversion as in [10]. Inert bacteria are obviously unable to divide, and for the sake of the model, simply occupy space in the biofilm. In this model, persister bacteria are only able to become living bacteria; do not have the ability to die; and are unresponsive to the presence of both antimicrobial and nutrient. The term 'porosity' is used in the model as a term describing the lack of any of the aforementioned particulates [25]. That is, an area of high porosity has low concentrations of the bacteria populations and EPS; conversely, an area of low porosity has high concentrations of one or all of the bacteria populations and EPS.

There are two soluble components incorporated into the model, nutrient and antimicrobial which are separate from the particulate components similar to [16]. In this paper, the antimicrobial is delivered in one of two ways: either as a liquid acting through diffusion from the surface of the biofilm, or through nanospheres which are present in the interior of the biofilm; the nanosphere subsequently degrades releasing antimicrobial. In order to describe the degradation of nanospheres, theory elucidated in Hopfenberg is utilized [26].

While a variety of methods have been used to mathematically describe the intricate processes involved in biofilm growth and decay, it is the modeller's goal to avoid oversimplification of the physical processes described while maintaining the intuitive appeal and utility of the model. The study presented here was done in an ef-

fort to combine the proposed methods of modeling a biofilm into one cohesive model. While the model seeks to describe a wide variety of processes in biofilm growth, it is desirable to maintain the simplicity of an algebraically solvable system of ordinary differential equations. In order to do this, several assumptions are made that do not compromise the accuracy of the model; these assumptions will be further discussed in this work. An initial biofilm model is proposed that does not contain these assumptions; however, the solution is described in detail for the subsequent simplified model. Among other simplifications, the biofilm is modeled as being one-dimensional and thus uniform in all directions except that which is perpendicular to the substratum on which the biofilm grows similar to [1, 19]. Also, many of the processes are decoupled by considering the multiple time scales during which they occur as seen in [27]. Further simplifications will be explained in the body of this work. In brief, all solutions presented here are for the case of a one-dimensional model, with infinite diffusion of antimicrobial, non-leaky boundaries for nutrient and antimicrobial, and lack of detachment of biofilm particulates. Since the model presented here can be solved almost completely algebraically, the systems will be solved utilizing the algebraic capabilities of MAPLE 10. A numerical scheme is utilized in the derivation of the height of the biofilm, but its computational form is easily computed on MAPLE 10 as well. It is hoped with this highly intuitive model of biofilm growth, and the breadth of processes explained herein, that one may better understand both the growth and decay of the biofilm, and its interaction with soluble components such as nutrient and antimicrobials.

CHAPTER II

THE MODEL

In this chapter, the governing equations used to describe the mathematical model for biofilm growth and decay will be described. Following the description of these equations, the assumptions made to simplify these equations will be discussed.

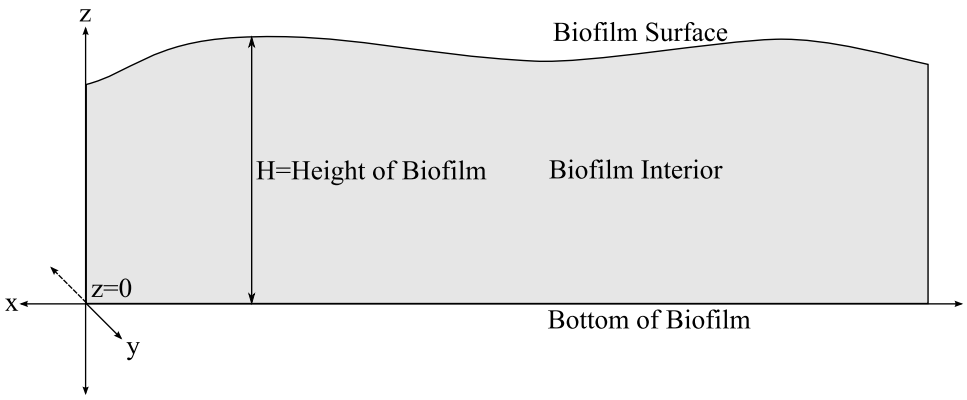

Figure 2.1: Representation of modelled biofilm geometry

2.1 Governing Equations

Biofilm growth is modelled by the equations that follow defined in the biofilm domain shown in figure 2.1.

Transportation and consumption of nutrient is given by

$$\phi S_t = \nabla \cdot (D_S \nabla S) - \mu_s \frac{S}{K_S + S} B, \tag{2.1}$$

where $\nabla = \frac{\partial}{\partial x}\hat{i} + \frac{\partial}{\partial z}\hat{k}$, for unit normal vectors $\hat{i}, \hat{k}$ in the horizontal and vertical directions respectively. S is the concentration of nutrient, ϕ represents the biofilm porosity, and diffusivity varies through biofilm according to $D_S = \phi^2 \bar{D}_S$, with $\bar{D}_S$ a constant. μ_S is a rate constant describing nutrient transfer into bacteria, K_S is the saturation level constant of nutrient, and B is the concentration of living bacteria. Note that (2.1) shows that the concentration of nutrient, S, is depleted by the living bacteria.

As mentioned in the introduction, there are three types of bacteria population concentrations included in the model: living bacteria denoted by B, inert bacteria denoted by B_i, and persister bacteria denoted by B_p. For each, the corresponding volume fraction, ϵ, and density, ρ (taken to be constant) are defined so that $B = \epsilon_B \rho_B$, $B_i = \epsilon_{B_i} \rho_{B_i}$, and $B_p = \epsilon_{B_p} \rho_{B_p}$ similar to [1, 28]. The advective velocity is denoted by $\vec{v}$. From this, the rate of transformation and transportation of living bacteria can be given by the following equation similar to [5]:

$$B_t + \nabla \cdot (\vec{v} B) = RHS_B, \tag{2.2}$$

where

$$RHS_B = \kappa_{growth} \mu_S \frac{S}{K_S + S} B - \kappa Y_S \mu_S C \frac{S}{K_S + S} B - b_{det}(z) B - bB - k_f B + k_R B_p. \tag{2.3}$$

Above, κ_{growth} (also denoted κ_g) represents the efficiency of bacteria converting nutrient into growth and κ represents the efficiency with which antimicrobial is converted into bacterial death. Note the first term represents an increase in living bacteria with time due to nutrient similar to [18]. In the second term, Y_S represents the yield coefficient for antimicrobial and C is the concentration of antimicrobial agent (mass per volume). This second term represents the depletion of living bacteria due to antimicrobial. The term $b_{det}(z)$ is the rate of bacterial detachment from the biofilm that varies directly with distance from the substratum as explained in [29], and leads to depletion of living bacteria in the biofilm via detachment. Bacterial detachment is elucidated in the literature [25]. The term b is the natural death rate of the bacteria species, which leads to decrease in living bacteria due to natural causes [1]. The term k_F is the rate of formation of persister cells from living cells and thus leads to depletion of living bacteria, and k_R is the reversion rate from persister cell to active living bacteria cell (from B_p to B) and thus brings about an increase in bacteria with time similar to [22].

Similarly, equations regarding how B_i and B_p vary with time can be written as follows:

$$B_{i_t} + \nabla \cdot (\vec{v} B_i) = RHS_{B_i}, \tag{2.4}$$

where

$$RHS_{B_i} = \kappa Y_S \mu_S C \frac{S}{K_S + S} B - b_{det}(z) B_i + bB. \tag{2.5}$$

Here the first term represents an increase in dead bacteria due to antimicrobial, the second term shows a decrease in the amount of dead bacteria present in the biofilm due to detachment, and the third term represents the increase in dead bacteria due to natural bacterial death. The equation for persister bacteria is given as

$$B_{p_t} + \nabla \cdot (\vec{v} B_p) = RHS_{B_p}, \tag{2.6}$$

where

$$RHS_{B_p} = k_F B - k_R B_p. \tag{2.7}$$

Notice, as mentioned previously, the persister bacteria cannot die without first transforming into living bacteria. This is in accordance with the theory that persister bacteria are in a non-respiring dormant state that is unresponsive to antimicrobial and nutrient [11, 22]. The absence of a detachment term in the equation for persisters is due to the belief that persister bacteria lie deep within the biofilm where detachment would be virtually impossible [10].

A final set of equations for the particulate components of the biofilm describes the formation and decay of EPS, or E in the biofilm. Similar to the three forms of bacteria described above, $E = \epsilon_E \rho_E$ where ρ_E is taken as a constant. How EPS varies with time is given by

$$E_t + \nabla \cdot (\vec{v} E) = RHS_E, \tag{2.8}$$

where

$$RHS_E = \kappa_{EPS} \mu_S \frac{S}{K_S + S} B - b_{det}(z) E. \tag{2.9}$$

Like living and dead bacteria, EPS can detach from the biofilm, leading to its depletion. Moreover, note that only living bacteria can produce EPS as indicated by the first term of equation (2.9). How the porosity of the biofilm varies in space and time is given by

$$\phi_t + \nabla \cdot (\vec{v}\phi) = RHS_\phi, \tag{2.10}$$

where

$$RHS_\phi = \frac{\phi}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right] + r_\phi. \tag{2.11}$$

Here, the term r_ϕ models changes in the porosity with respect to space and time due to influences besides the particulate populations similar to [25]. In [25], a term comparable to r_ϕ is used to provide a source for transformation processes occurring in the region of porosity.

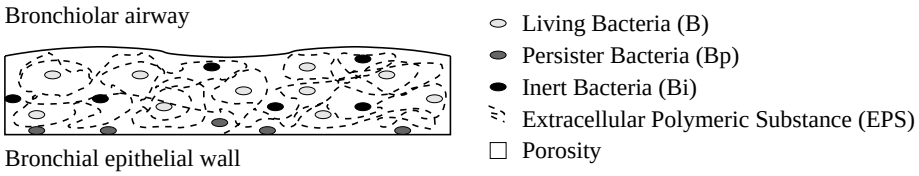

Figure 2.2: Representation of two dimensional model of biofilm with four biofilm constituents and porosity indicated

2.1.1 Advective velocity derivation

Now, the model assumes that the domain of the biofilm has no voids. It therefore follows that,

$$\phi + \epsilon_B + \epsilon_{B_i} + \epsilon_{B_p} + \epsilon_E = 1. \tag{2.12}$$

That is, the entire biofilm domain is comprised of the sum of the volume fractions as seen in [16, 19, 25, 30]. Notice that as the volume fractions for the particulate components decrease, the porosity increases allowing ease of transport for soluble components such as nutrient or antimicrobial.

From equation (2.12), and the definitions of the volume fractions, it follows that

$$\phi + \frac{B}{\rho_B} + \frac{B_i}{\rho_{B_i}} + \frac{B_p}{\rho_{B_p}} + \frac{E}{\rho_E} = 1. \tag{2.13}$$

Differentiating both sides of (2.13) with respect to time yields

$$\phi_t + \frac{B_t}{\rho_B} + \frac{B_{i_t}}{\rho_{B_i}} + \frac{B_{p_t}}{\rho_{B_p}} + \frac{E_t}{\rho_E} = 0. \tag{2.14}$$

By dividing each of (2.2), (2.4), (2.6), (2.8), and (2.10) by ρ_B, ρ_{B_i}, ρ_{B_p}, ρ_E and 1 respectively the following equations result:

Column 1 Column 2

$$\begin{aligned}
&\overbrace{\frac{B_t}{\rho_B}} + \overbrace{\nabla \cdot \left(\vec{v}\frac{B}{\rho_B}\right)} = \frac{RHS_B}{\rho_B},\\
&\frac{B_{i_t}}{\rho_{B_i}} + \nabla \cdot \left(\vec{v}\frac{B_i}{\rho_{B_i}}\right) = \frac{RHS_{B_i}}{\rho_{B_i}},\\
&\frac{B_{p_t}}{\rho_{B_p}} + \nabla \cdot \left(\vec{v}\frac{B_p}{\rho_{B_p}}\right) = \frac{RHS_{B_p}}{\rho_{B_p}},\\
&\frac{E_t}{\rho_E} + \nabla \cdot \left(\vec{v}\frac{E}{\rho_E}\right) = \frac{RHS_E}{\rho_E},\\
&\phi_t + \nabla \cdot (\vec{v}\phi) = \frac{\phi}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right] + r_\phi.
\end{aligned}$$

Summing the above equations column-wise yields

$$0 + \nabla \cdot \vec{v} = \frac{1}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right] + r_\phi,$$

where column one was simplified by factoring out the differential operator $\frac{\partial}{\partial t}$ and using (2.13) and column two was simplified by using (2.12). The last equation can be simplified further giving,

$$\nabla \cdot (\vec{v}) = \frac{1}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right] + r_\phi. \tag{2.15}$$

Thus the biofilm particulates cause advection in biofilm to occur and result in a velocity of the biofilm front similar to [31]. Notice that equations (2.10) and (2.11) with ϕ constant would be equivalent to (2.15), if r_ϕ were not prescribed. Thus as previously explained, r_ϕ is included to provide a means to describe the variation in the porosity with space and time, so that ϕ can be some changing quantity.

Darcy's Law states that $\vec{v} = -\lambda \nabla P$, where $\lambda(\phi)$ is the Darcy parameter which represents the permeability of the medium [18] and P is pressure. Here, Darcy's Law is applied since the biofilm is assumed to be a composed of porous media. In order to find the advective velocity the following Poisson equation

$$-\nabla \cdot (\lambda \nabla P) = \frac{1}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right] + r_\phi \tag{2.16}$$

must be solved subject to Neumann conditions on the bottom and sides of the biofilm (thus the velocity of biofilm constituents coming in and out of the biofilm boundary is prescribed) while maintaining continuity with the airway pressure at the boundary between the biofilm and bronchial epithelial wall, shown in figure 2.2.

2.1.2 Antimicrobial Agent

The expression for the release rate of the agent from a single nanoparticle was derived using the Hopfenberg model [26]. Here, a nanoparticle refers to a sphere on the scale of nanometers (called a nanosphere), that contains a determined amount of

antimicrobial. Hopfenberg explains that the amount of antimicrobial that would be released from a nanosphere in time is given by,

$$M(t) = C_0 \left[\frac{4}{3}\pi R^3 - \frac{4}{3}\pi r(t)^3 \right], \tag{2.17}$$

where R represents the radius of the nanosphere initially, $r(t)$ represents the changing radius of the nanosphere as it degrades with time, and C_0 represents the concentration of antimicrobial within the nanosphere. Note that $r(0) = R$, and $r\left(\frac{C_0 R}{k}\right) = 0$. To determine the rate of release of antimicrobial agent, take the derivative with respect to time of (2.17) yielding:

$$\frac{dM(t)}{dt} = -C_0 4\pi r(t)^2 \frac{dr}{dt}. \tag{2.18}$$

Now the mass released from the nanosphere in time is assumed to be linearly dependent on the surface area of the nanosphere with a rate constant k. That is, it is assumed that $\frac{dM(t)}{dt} = k4\pi r^2$. Thus from equation (2.18) it is apparent that $k = -C_0 \frac{dr}{dt}$ which implies

$$\frac{dr}{dt} = \frac{-k}{C_0}. \tag{2.19}$$

Integrating (2.19) with respect to time and applying the initial condition $r(0) = R$ yields,

$$r(t) = R - \frac{kt}{C_0}. \tag{2.20}$$

From (2.18), (2.19) and (2.20), the rate of release of antimicrobial from the nanosphere is given as

$$\frac{dM(t)}{dt} = \left\{ \begin{array}{cc} 4\pi k \left[R - \frac{kt}{C_0}\right]^2, & 0 \leq t \leq \frac{C_0 R}{k} \\ 0, & t > \frac{C_0 R}{k} \end{array} \right\}. \tag{2.21}$$

Factoring out R in (2.21) yields the following equation explaining the release rate of antimicrobial:

$$\frac{dM(t)}{dt} = \left\{ \begin{array}{cc} 4\pi R^2 k \left[1 - \frac{kt}{C_0 R}\right]^2, & 0 \leq t \leq \frac{C_0 R}{k} \\ 0, & t > \frac{C_0 R}{k} \end{array} \right\}. \tag{2.22}$$

Thus, a governing equation for the transportation and reactivity of the antimicrobial agent within the biofilm is described by,

$$\phi C_t = \nabla \cdot (\phi D_C \nabla C) + \delta(\vec{x} - \vec{x_0}) \left\{ \begin{array}{cc} 4\pi R^2 k \left[1 - \frac{kt}{C_0 R}\right]^2, & 0 \leq t \leq \frac{C_0 R}{k} \\ 0, & t > \frac{C_0 R}{k} \end{array} \right\} - k_C C. \tag{2.23}$$

Similar to (2.1), diffusivity of the antimicrobial within the biofilm varies with porosity as $D_C = \phi \bar{D}_C$, where D_C is a constant. Notice the expression for the release rate of the antimicrobial from a single nanoparticle just derived using the Hopfenberg model [26] in the right side of equation (2.23). Also, in (2.23) a delta function is used to represent the location $\vec{x_0}$ of the antimicrobial agent and k_C represents the rate at which the antimicrobial is lost from the domain of the biofilm which is assumed to be a first order reaction of loss.

2.1.3 Height of biofilm

The height or thickness of the biofilm is given by,

$$\frac{dH(\vec{x},t)}{dt} = \hat{n}\cdot\vec{v} - v_{det} = -\hat{n}\cdot\lambda\nabla P - \frac{k_{det}H^2}{E}, \tag{2.24}$$

which is a kinematic condition for the biofilm interface $H(\vec{x},t)$. Here, $\hat{n}\cdot\vec{v}$, represents the normal speed of the front component of the advective velocity, moving in the direction normal to the surface of the biofilm similar to [19]. Notice in (2.24), the detachment from the biofilm increases as the biofilm height increases similar to [16, 25, 32], and decreases with the addition of more EPS or E, which provides structure for the biofilm.

2.1.4 Boundary conditions for Antimicrobial and Nutrient

Robin boundary conditions are applied at the surface of the biofilm (see figures 2.1, 2.2):

$$-\phi D_C \nabla C\cdot\hat{n} = k_{SC}\left[C - C_{source}\right] \tag{2.25}$$

and

$$-\phi D_S \nabla S\cdot\hat{n} = k_{SS}\left[S - S_{source}\right]. \tag{2.26}$$

For both (2.25) and (2.26), the left sides of the equations account for the transport by diffusion across the barrier, and the right sides account for transport by convection-fluid motion (the negative sign is in place since $S - S_{source}$ is negative and yet the gradient is positive). Here, $\hat{n}$ is the unit outward normal pointing out of the biofilm surface, away from the biofilm interior. k_{SC} and k_{SS} are the diffusivity constants

for the surface of the biofilm for antimicrobial and nutrient respectively; C_{source}, and S_{source} are prescribed concentrations of antimicrobial and nutrient, respectively, applied to the surface of the biofilm. Note also that $D_C = \phi \bar{D_C}$ and $D_S = \phi \bar{D_S}$. Similar to equations (2.25) and (2.26), boundary conditions exist for the interface between the epithelial tissue and the biofilm, where C_{source} and S_{source} are replaced with C_{tissue} and S_{tissue}, accounting for the concentration of antimicrobial and nutrient at the bronchial epithelium-biofilm interface. The unit normal vector, $\hat{n}$, is assumed to point away from the biofilm and into the epithelial tissue.

The above model can be used to find effectiveness of antimicrobial treatment options, optimal dosing, and durations of treatment. At time $t = 0$, $C = 0$ and values are prescribed for S, E, ϕ, B, B_p, and B_i.

2.2 Assumptions for Simplification to 1-D Model

A one-dimensional model is derived from the above two-dimensional model. This section outlines the assumptions made to simplify the two-dimensional model.

First, the porosity of the biofilm, ϕ, is assumed to be a known constant which implies that $r_\phi = 0$ similar to [1, 31]. This is different from many models that assume non-constant porosity [25, 29]. For this reason, it immediately follows that equations (2.10) and (2.11) can be ignored, as they discuss how porosity varies in space and time.

Second, a thin domain approximation is made assuming that the average depth of the biofilm, $\tilde{d}$, is much less than the wavelength of the periodic shape of the

biofilm, $\tilde{\lambda}$ (Figure 2.3). Defining $\vec{w}$ and $\vec{u}$ as the respective vertical and horizontal components of the velocity $\vec{v}$, it follows that,

$$\vec{u} = -\lambda \frac{\partial P}{\partial x} \implies \vec{u}_{avg} = -\lambda \frac{P_{avg}}{\tilde{\lambda}}. \tag{2.27}$$

Likewise,

$$\vec{w}_{avg} = -\lambda \frac{P_{avg}}{\tilde{d}}. \tag{2.28}$$

So since $\frac{1}{\tilde{\lambda}} \ll \frac{1}{\tilde{d}}$, it follows that $\frac{\partial}{\partial x} \ll \frac{\partial}{\partial z}$ and $\vec{u}_{avg} \ll \vec{w}_{avg}$. Hence H is flat and simply a function of time. This thin domain approximation implies that all derivatives with respect to the horizontal axis, x, can be taken as zero, since they are so small when compared to those changing in the vertical axis, z.

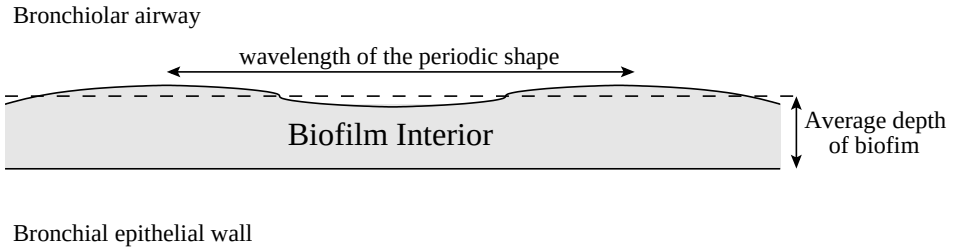

Figure 2.3: Depiction of thin domain approximation showing the average depth of biofilm, $\tilde{d}$, is much less than the wavelength of the periodic shape of the biofilm, $\tilde{\lambda}$

Equation (2.1) is rewritten so that the nutrient quantity no longer depends upon the bacteria population that changes with time but rather the nutrient concentration depends upon the average size of the bacterial population; as a result of the above assumptions and approximations, (2.1) becomes,

$$\phi D_S S_{zz} - k_S S = 0, \tag{2.29}$$

where $D_S = \phi \bar{D_S}$ and ϕ and $\bar{D_S}$ are constants. In order to decouple B from S it is assumed that $k_S \approx \frac{\mu_S}{K_S} B_{avg}$.

A third assumption is that values for ρ_B, ρ_{B_i}, ρ_{B_p} and ρ_E are large when compared to RHS_B, RHS_{B_i}, RHS_{B_p}, and RHS_E in equation (2.15). As a result of this assumption, and the assumption above that $r_\phi = 0$, the quantity $\nabla \cdot (\vec{v})$ can be taken as zero for equation (2.15). Now if $\nabla \cdot (\vec{v}) = \frac{\partial}{\partial x} u + \frac{\partial}{\partial z} w \approx 0$ and since $\frac{\partial}{\partial x} u$ can be eliminated as a result of the thin domain approximation, then $\frac{\partial}{\partial z} w$ can be eliminated from equations (2.2), (2.4), (2.6), and (2.8) (note that $\nabla \cdot (\vec{v} \diamondsuit) = \frac{\partial}{\partial x}(\diamondsuit u) + \frac{\partial}{\partial z}(\diamondsuit w)$ for $\diamondsuit = B, B_i, B_p$, or E).

A fourth modification made to the original model to decouple equations was the removal of detachment terms in equations (2.3), (2.5), (2.9) and (2.24).

With these modifications, equations (2.2), (2.3), (2.4), (2.5), (2.6), (2.7) (2.8), (2.23), and (2.24) can be rewritten as follows for the one-dimensional model:

$$B_t = \kappa_{growth} \mu_S \frac{S}{K_S + S} B - \kappa Y_S \mu_S C \frac{S}{K_S + S} B - bB - k_f B + k_R B_p - \alpha, \tag{2.30}$$

$$B_{i_t} = \kappa Y_S \mu_S C \frac{S}{K_S + S} B + bB, \tag{2.31}$$

$$B_{p_t} = k_f B - k_R B_p, \tag{2.32}$$

$$E_t = \kappa_{EPS} \mu_S \frac{S}{K_S + S} B, \tag{2.33}$$

$$w_z = \frac{\alpha}{1 - \phi}, \text{ where } \alpha = \frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}, \tag{2.34}$$

defining RHS_B, RHS_{B_i}, RHS_{B_p}, and RHS_E as the right sides of equations (2.30), (2.31), (2.32), and (2.33) respectively. Also,

$$\phi C_t - \phi D_C C_{zz} = \delta(z - z_0) \left\{ \begin{array}{cc} 4\pi R^2 k \left[1 - \frac{kt}{C_0 R}\right]^2, & 0 \leq t \leq \frac{C_0 R}{k} \\ 0, & t > \frac{C_0 R}{k} \end{array} \right\}, \tag{2.35}$$

and

$$\frac{dH}{dt} = w. \tag{2.36}$$

In defining (2.35) from equation (2.23), k_C is not included to eliminate the role of antimicrobial being lost from the biofilm domain. The α term in equations (2.30), (2.31), and (2.34) is required to maintain conservation of volume. That is, α is included to maintain that the biofilm is not comprised of void as asserted in subsection 2.1.1. The term α will be fully derived in the following subsection. The inclusion of α provides a means for the modelled biofilm to react to availability of volume similar to other models that include substrate availability as a constraint [1].

2.2.1 Derivation of α for Conservation of Volume

As explained above, the term α must be used to maintain conservation of volume. To clarify, consider rewriting equations (2.2), (2.4), (2.6), (2.8), as follows:

$$B_t + \frac{\partial}{\partial x}(uB) + \frac{\partial}{\partial z}(wB) = RHS_B, \tag{2.37}$$

$$B_{i_t} + \frac{\partial}{\partial x}(uB_i) + \frac{\partial}{\partial z}(wB_i) = RHS_{B_i}, \tag{2.38}$$

$$B_{p_t} + \frac{\partial}{\partial x}(uB_p) + \frac{\partial}{\partial z}(wB_p) = RHS_{B_p}, \tag{2.39}$$

$$E_t + \frac{\partial}{\partial x}(uE) + \frac{\partial}{\partial z}(wE) = RHS_E. \tag{2.40}$$

By the thin domain approximation the terms in the equations above containing horizontal velocity component u can be ignored giving us the following modified equations:

$$B_t + \frac{\partial}{\partial z}(wB) = RHS_B, \tag{2.41}$$

$$B_{i_t} + \frac{\partial}{\partial z}(wB_i) = RHS_{B_i}, \tag{2.42}$$

$$B_{p_t} + \frac{\partial}{\partial z}(wB_p) = RHS_{B_p}, \tag{2.43}$$

$$E_t + \frac{\partial}{\partial z}(wE) = RHS_E. \tag{2.44}$$

As a result of the third assumption listed in subsection 2.2, the terms, $\frac{\partial}{\partial z}(wB)$, $\frac{\partial}{\partial z}(wB_i)$, $\frac{\partial}{\partial z}(wB_p)$, and $\frac{\partial}{\partial z}(wE)$ are removed from the left side of equations (2.41), (2.42), (2.43), (2.44) respectively. Though this assumption simplifies the solution of the system, it causes the system to no longer be constrained by conservation of volume.

To elucidate this concept, consider dividing equations (2.41), (2.42), (2.43), (2.44), by their respective densities. This yields:

$$\frac{B_t}{\rho_B} + \frac{\frac{\partial}{\partial z}(wB)}{\rho_B} = \frac{RHS_B}{\rho_B}, \tag{2.45}$$

$$\frac{B_{i_t}}{\rho_{B_i}} + \frac{\frac{\partial}{\partial z}(wB_i)}{\rho_{B_i}} = \frac{RHS_{B_i}}{\rho_{B_i}}, \tag{2.46}$$

$$\frac{B_{p_t}}{\rho_{B_p}} + \frac{\frac{\partial}{\partial z}(wB_p)}{\rho_{B_p}} = \frac{RHS_{B_p}}{\rho_{B_p}}, \tag{2.47}$$

$$\frac{E_t}{\rho_E} + \frac{\frac{\partial}{\partial z}(wE)}{\rho_E} = \frac{RHS_E}{\rho_E}. \tag{2.48}$$

Also, as part of the derivation, consider equation (2.10), which can be rewritten to ignore the horizontal velocity, and explicitly defining RHS_ϕ (excluding the term r_ϕ since it equals 0 as discussed in the first assumption of subsection 2.2):

$$\phi_t + (w\phi)_z = \frac{\phi}{1-\phi}\left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right]. \tag{2.49}$$

Summing equations (2.45), (2.46), (2.47), (2.48), and (2.49) and grouping related terms yields the following:

$$\left[\frac{B_t}{\rho_B}+\frac{B_{i_t}}{\rho_{B_i}}+\frac{B_{p_t}}{\rho_{B_p}}+\frac{E_t}{\rho_E}+\phi_t\right]+\left[\frac{(wB)_z}{\rho_B}+\frac{(wB_i)_z}{\rho_{B_i}}+\frac{(wB_p)_z}{\rho_{B_p}}+\frac{(wE)_z}{\rho_E}+(w\phi)_z\right]$$
$$=\left[\frac{RHS_B}{\rho_B}+\frac{RHS_{B_i}}{\rho_{B_i}}+\frac{RHS_{B_p}}{\rho_{B_p}}+\frac{RHS_E}{\rho_E}\right]$$
$$+\frac{\phi}{1-\phi}\left[\frac{RHS_B}{\rho_B}+\frac{RHS_{B_i}}{\rho_{B_i}}+\frac{RHS_{B_p}}{\rho_{B_p}}+\frac{RHS_E}{\rho_E}\right]. \quad (2.50)$$

Define the quantity,

$$\alpha=\left[\frac{RHS_B}{\rho_B}+\frac{RHS_{B_i}}{\rho_{B_i}}+\frac{RHS_{B_p}}{\rho_{B_p}}+\frac{RHS_E}{\rho_E}\right].$$

Thus equation (2.50) can be rewritten as,

$$\begin{aligned}\left[\frac{B_t}{\rho_B}+\frac{B_{i_t}}{\rho_{B_i}}+\frac{B_{p_t}}{\rho_{B_p}}+\frac{E_t}{\rho_E}+\phi_t\right] &+ \left[\frac{(wB)_z}{\rho_B}+\frac{(wB_i)_z}{\rho_{B_i}}+\frac{(wB_p)_z}{\rho_{B_p}}+\frac{(wE)_z}{\rho_E}+(w\phi)_z\right]\\ &= \alpha+\frac{\phi}{1-\phi}\alpha\\ &= \frac{1-\phi}{1-\phi}\alpha+\frac{\phi}{1-\phi}\alpha\\ &= \frac{\alpha}{1-\phi}. \quad (2.51)\end{aligned}$$

By the rules of differentiation,

$$\left[\frac{B_t}{\rho_B}+\frac{B_{i_t}}{\rho_{B_i}}+\frac{B_{p_t}}{\rho_{B_p}}+\frac{E_t}{\rho_E}+\phi_t\right]=\frac{\partial}{\partial t}\left(\frac{B}{\rho_B}+\frac{B_i}{\rho_{B_i}}+\frac{B_p}{\rho_{B_p}}+\frac{E}{\rho_E}+\phi\right). \quad (2.52)$$

Recall, the sum of all of the volume fractions of the constituents of the biofilm must equal unity [16, 25]. That is,

$$\left(\frac{B}{\rho_B}+\frac{B_i}{\rho_{B_i}}+\frac{B_p}{\rho_{B_p}}+\frac{E}{\rho_E}+\phi\right)=1. \quad (2.53)$$

Thus,

$$\left[\frac{B_t}{\rho_B}+\frac{B_{i_t}}{\rho_{B_i}}+\frac{B_{p_t}}{\rho_{B_p}}+\frac{E_t}{\rho_E}+\phi_t\right]=\frac{\partial}{\partial t}(1)=0. \quad (2.54)$$

Substituting equations (2.54) into (2.51) yields the following:

$$\left[\frac{(wB)_z}{\rho_B} + \frac{(wB_i)_z}{\rho_{B_i}} + \frac{(wB_p)_z}{\rho_{B_p}} + \frac{(wE)_z}{\rho_E} + (w\phi)_z\right] = \frac{\alpha}{1-\phi}. \tag{2.55}$$

Again, by the rules of differentiation and (2.53), the following is obtained:

$$w_z = \frac{\alpha}{1-\phi}. \tag{2.56}$$

Thus, if one were to assume that the terms $\frac{(wB)_z}{\rho_B}$, $\frac{(wB_i)_z}{\rho_{B_i}}$, $\frac{(wB_p)_z}{\rho_{B_p}}$, and $\frac{(wE)_z}{\rho_E}$ were insignificant in equations (2.41), (2.42), (2.43) and (2.44), the term $w_z(1-\phi)$ would be neglected. To illustrate, consider:

$$\begin{aligned} \frac{(wB)_z}{\rho_B} + \frac{(wB_i)_z}{\rho_{B_i}} + \frac{(wB_p)_z}{\rho_{B_p}} + \frac{(wE)_z}{\rho_E} &= w_z\left[\frac{(B)_z}{\rho_B} + \frac{(B_i)_z}{\rho_{B_i}} + \frac{(B_p)_z}{\rho_{B_p}} + \frac{(E)_z}{\rho_E}\right] \\ &= w_z(1-\phi). \end{aligned} \tag{2.57}$$

As seen in (2.56), $w_z = \frac{\alpha}{1-\phi}$, which implies that the neglected term is α. As a result, α is added to the sum of equations (2.41), (2.42), (2.43) and (2.44) to abide by the laws of conservation. In order to restrict the unbounded growth of EPS and living bacteria in the system, yet simplify the system to be solved by ordinary differential equation solution techniques, the quantity α is placed into equation (2.41). This explains the presence of the term $\alpha = \left[\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right]$ in equation (2.30) defined in subsection 2.2.

Physically, the reason for the presence of α in (2.30) is because the living bacteria within the biofilm would be the form that would be constrained by volume limitations. Other models propose conservation of biofilm resources as constraining factors [1, 6] on living bacteria populations. As a result, the assumption that the

living bacteria would have slowed growth due to spatial limitations is consistent with living bacteria being constrained by limited biofilm resources.

CHAPTER III

THE SOLUTION PROCEDURE

In this chapter, the procedure used to solve the equations described in the one-dimensional model will be fully explained. Note that this procedure works with the one-dimensional model that has been derived from the higher dimensional model.

3.1 Multiple Time-scale Analysis

In order to solve many of the ordinary differential equations in the model, multiple time-scales are exploited so that some quantities that change with respect to time are considered to be constant similar to [27]. Though many quantities in this model vary with time, they do so at very different rates. For this reason, those quantities that change very slowly when compared to a sought quantity can be considered constant to ease the calculation of a sought quantity. The processes involved in the model listed from fastest to slowest are: diffusion of soluble species (nutrient and antimicrobial); growth and decay of particulate species; degradation of nanoparticles and hence the release of antimicrobial; and the growth and decay of biofilm height.

3.2 Solution Techniques

This section explains the techniques for solving the simplified one-dimensional model with use of multiple time-scales. There are two solutions: one for antimicrobial delivery to the surface of the biofilm, and one for antimicrobial delivery to the biofilm using nanospheres. For both, the solution is done in two parts: the first part of the solution allows the biofilm to grow without the presence of antimicrobial and the second part of the solution uses the final conditions of the first solution as its initial conditions and allows the biofilm to interact in the presence of antimicrobial. The setup for both parts of the solution is the same; only the values for prescribed constants and initial conditions are different.

3.2.1 Antimicrobial Applied at Surface

Both parts in this subsection explain solutions for the case of constant antimicrobial applied to the surface of the biofilm. The first part is the solution for biofilm growth when antimicrobial is not present. The second part is the solution for biofilm growth after the biofilm has grown, and is in the presence of antimicrobial.

Part One: Growth Before Antimicrobial is Present

First, put equations (2.30), (2.31), and (2.32) into matrix form, as they are coupled. In the matrix, the terms CC and SS take the place of the terms $C(z)$ and $S(z)$ and will be replaced later with the solutions of $C(z)$ and $S(z)$ as a result of the faster time-scale with which the antimicrobial and nutrient are assumed to diffuse.

In essence, since the antimicrobial and nutrient diffuse so quickly, their concentration is virtually constant when viewed from the vantage of the other processes occurring in the biofilm outlined in section 3.1. The equations in matrix form are written as:

$$\begin{bmatrix} B_t \\ B_{it} \\ B_{pt} \end{bmatrix} = \begin{bmatrix} A_{11} & A_{12} & A_{13} \\ A_{21} & A_{22} & A_{23} \\ A_{31} & A_{32} & A_{33} \end{bmatrix} \begin{bmatrix} B \\ B_i \\ B_p \end{bmatrix},$$

where

$$\begin{aligned}
A_{11} &= \kappa_g \mu_S \frac{S}{K_S + S} - \kappa Y_S \mu_S C \frac{S}{K_S + S} - b - k_f - \\
&\quad \left(\frac{\kappa_g \mu_S \frac{S}{K_S+S} - \kappa Y_S \mu_S C \frac{S}{K_S+S} - b - k_f}{\rho_B} + \frac{\kappa Y_S \mu_S C \frac{S}{K_S+S} + b}{\rho_{B_i}} + \right. \\
&\quad \left. \frac{k_f}{\rho_{B_p}} + \frac{\kappa_{EPS} \mu_S \frac{S}{K_S+S}}{\rho_E} \right) \rho_B, \\
A_{12} &= 0, \\
A_{13} &= k_R - \left(\frac{k_R}{\rho_B} - \frac{k_R}{\rho_{B_p}} \rho_B \right) \rho_B, \\
A_{21} &= \kappa Y_S \mu_S C \frac{S}{K_S + S} + b, \\
A_{22} &= 0, \\
A_{23} &= 0, \\
A_{31} &= k_f, \\
A_{32} &= 0, \qquad \text{and} \\
A_{33} &= -k_R.
\end{aligned}$$

Next, find the eigenvalues and eigenvectors of this matrix. For convenience name the eigenvalues $\lambda_1, \lambda_2, \lambda_3$ and the eigenvectors $\vec{v_1}$, $\vec{v_2}$, $\vec{v_3}$. Place the eigenvectors

into a matrix called L. Thus,

$$L = \begin{bmatrix} v_{1,1} & v_{2,1} & v_{3,1} \\ v_{1,2} & v_{2,2} & v_{3,2} \\ v_{1,3} & v_{2,3} & v_{3,3} \end{bmatrix},$$

where $v_{i,j}$ represents the jth component of the ith vector ($j = 1$, $j = 2$, and $j = 3$ correspond to the B, B_i, and B_p components respectively). Then take the inverse of the matrix L and call it M, so that

$$M = L^{-1} = \begin{bmatrix} v_{1,1} & v_{1,2} & v_{1,3} \\ v_{2,1} & v_{2,2} & v_{2,3} \\ v_{3,1} & v_{3,2} & v_{3,3} \end{bmatrix}.$$

Now set up a matrix called Q composed of B_0, B_{i0}, and B_{p0}, which are the respective initial concentrations of living bacteria, inert bacteria, and persister bacteria. Thus

$$Q = \begin{bmatrix} B_0 \\ B_{i0} \\ B_{p0} \end{bmatrix}.$$

In order to find the integrating constants, c_1, c_2, and c_3, multiply matrices M and Q, and call this matrix P. That is, $P{=}M * Q$. The integrating constants are defined by taking components of the P matrix since

$$P = \begin{bmatrix} c_1 \\ c_2 \\ c_3 \end{bmatrix}.$$

After this is done, all the constants found in either table A.1 or table A.2 can be defined (depending on the size of initial persister bacteria population being considered). These tables can be found in appendix A.

Now, equations can be built for $B(t)$, $B_i(t)$, and $B_p(t)$. They are defined as:

$$B(t) = c_1 v_{1,1} e^{\lambda_1 t} + c_2 v_{2,1} e^{\lambda_2 t} + c_3 v_{3,1} e^{\lambda_3 t}, \tag{3.1}$$

$$B_i(t) = c_1 v_{1,2} e^{\lambda_1 t} + c_2 v_{2,2} e^{\lambda_2 t} + c_3 v_{3,2} e^{\lambda_3 t}, \tag{3.2}$$

and

$$B_p(t) = c_1 v_{1,3} e^{\lambda_1 t} + c_2 v_{2,3} e^{\lambda_2 t} + c_3 v_{3,3} e^{\lambda_3 t}, \tag{3.3}$$

since they are solutions to the system of ordinary differential equations (ODEs) defined by the matrix in the first step of the solution procedure.

Next, substitute the solution for $B(t)$ obtained above in equation (3.1) and then solve the ordinary differential equation (ODE) for E seen in equation (2.33) with the initial condition $E(0) = E_0$. The resulting solution to the ODE is named $E(t)$.

The equation for concentration of nutrient, S, should then be solved, and stored as $S(z)$. That is, solve the ODE

$$\phi^2 \bar{D_S} S_{zz} - k_S S = 0, \tag{3.4}$$

subject to the boundary condition at $z = 0$ that assumes no flux at the bottom of the biofilm

$$S_z(0) = 0, \tag{3.5}$$

and boundary condition at the top of the biofilm that assumes there is a source for nutrient in the airway of the bronchi

$$-\phi^2 \bar{D}_S S(d)_z = k_{HS}(S(d) - S_{Source}). \tag{3.6}$$

In the second boundary condition, d acts as a placeholder for the height of the biofilm. The height of the biofilm will later be substituted into this placeholder once the height equation is solved.

Following this solution, one would find the solution to the ODE for the concentration of antimicrobial in the biofilm, seen in equation (2.35). In the first part of the solution however it is assumed that there is no antimicrobial present and the solution for antimicrobial concentration is unnecessary, as the antimicrobial is effectively rendered powerless since $\kappa = 0$ as shown in both table A.1 and table A.2 (recall: κ indicates the effectiveness of the antimicrobial).

Next, the solutions for $C(z)$ and $S(z)$ must respectively replace the temporary variables of CC and SS used in defining $B(t)$, $B_i(t)$, $B_p(t)$ and $E(t)$. Upon doing this, $B(t)$, $B_i(t)$, $B_p(t)$ and $E(t)$ are algebraic functions of z and t. As previously stated, the solutions for $C(z)$ and $S(z)$ can now be replaced into the equations for $B(t)$, $B_i(t)$, $B_p(t)$ and $E(t)$ due to our time-scale assumption that the diffusion of

nutrient and antimicrobial is very fast compared to the other processes occurring in the biofilm growth and decay.

Now, the advective velocity, w, can be found by integrating both sides of equation (2.34). Define the multi-variable function

$$w(\zeta, t, d) = \int_0^{\zeta} \frac{\left(\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E}\right)}{1-\phi} \mathrm{d}z, \tag{3.7}$$

where ζ represents some location along the vertical axis of the one-dimensional biofilm, and t represents time. As before, d represents a placeholder for the height of the biofilm. The presence of d in the right hand side of equation (3.7) is a result of the nutrient equation being substituted into the temporary variable SS. Thus $w(\zeta, t, d)$ is a function of three variables. Nonetheless, since both ζ and d represent the height of the biofilm for any time t, in practice the same quantity representing height will be substituted simultaneously in for ζ and d.

Next calculate the height of the biofilm at a given time through a simple numerical integration scheme. To do so, define a value for Δt which is the time-step, and a value for mm which is the total number of steps in the partition for the time period where height is being calculated. That is, $mm = \frac{t_{med}}{\Delta t}$ for the first part of the solution where antimicrobial is not yet present, since t_{med} represents the time when the antimicrobial is applied. Configure two arrays, one for the values that exist in the time domain called XX (here, this refers to the time values in days of the 3 days of growth where no antimicrobial is present), and another for those values in the range representing height of biofilm before antimicrobial is added called $HH1$ so that

each array contains elements from 1 to mm+1. Initialize the $HH1$ array by defining $HH1[1]$ to some initial height value; call it h_0.

To find this initial height value consider that for the data presented here, the porosity remains constant; that is, $\phi = .995$. According to table A.1 and table A.2 seen in appendix A, $\rho_B = \rho_{B_p} = 0.2\frac{g}{cm^3}$. By equation (2.13), and given that the initial concentrations of inert bacteria and EPS are zero ($B_{i0} = E_0 = 0$), it follows that,

$$\frac{B_0}{0.2} + \frac{B_{p0}}{0.2} = .005. \tag{3.8}$$

The concentration of living bacteria in the biofilm is given by,

$$B_0 = \frac{\eta_B}{h_0} B^*, \tag{3.9}$$

where η_B is the number of bacteria in the biofilm initially, h_0 is the initial height of the biofilm of volume 1 cm $\times$ 1 cm $\times$ h_0 and B^* is the mass of a single living bacteria. Similarly, the concentration of persister bacteria in the biofilm is given by,

$$B_{p0} = \frac{\eta_{B_p}}{h_0} {B_p}^*. \tag{3.10}$$

By fixing η_B and η_{B_p} for a particular run of the model, together with equations (3.8), (3.9) and (3.10), and noting that $B^* = {B_p}^*$, the initial height is determined as:

$$h_0 = \frac{B^*}{0.001}(\eta_B + \eta_{B_p}). \tag{3.11}$$

Subsequently, one can substitute this value for h_0 into equations (3.9) and (3.10) to determine the initial concentrations of living and persister bacteria desired for a given run of the model. Thus, by choosing starting population sizes for the persisters and living bacteria, one can maintain a constant porosity and more easily compare the results presented from various runs of the model.

Now to find H, iterate a variable i from 1 to mm, and define

$$HH1[i+1] = HH1[i] + w(HH1[i], i * \Delta t, HH1[i]) * \Delta t. \tag{3.12}$$

This provides the height for the biofilm at varying times from 0 to t_{med}. For use in the second part of the solution, define $H_{final} = HH1[mm+1]$, which is the height of the biofilm at the last point in time before antimicrobial is applied. Output the values for the height array before antimicrobial is applied with its corresponding time values by setting up a two dimensional array called $HeightBeforeMed$ and use the scheme that follows. Iterate m from 1 to $mm+1$ and compute the first dimension of the array representing time values for the period of time between $t = 0$ and $t = t_{med}$:

$$HeightBeforeMed[m, 1] = \frac{(m-1) * tmeddays}{mm}.$$

Likewise, iterate m from 1 to $mm+1$ and compute the second dimension of the array representing height values:

$$HeightBeforeMed[m, 2] = HH1[m].$$

After calculating the height, the mass of any group of components in the biofilm at a given time can be calculated. To illustrate the process, consider finding

the mass of living and growing bacteria. To find the total mass of living bacteria in the entire thickness of the biofilm, define the three-variable function,

$$fB(\zeta, t, d) = \int_0^{\zeta} B(t)\,\mathrm{d}z, \tag{3.13}$$

where ζ corresponds to a location in the one-dimensional biofilm. Again, d represents the height of the biofilm and is present in the right hand side implicitly through the substitution of the solution to the nutrient equation into the equation for concentration of bacteria. The function fB previously specified will determine the total mass of bacteria from the base of the biofilm to the given location ζ per cubic centimeter, since B(t) gives the concentration of bacteria (g/cm^3) at time t at a given location in the vertical axis, z. To clarify the calculation of fB consider the unit analysis. When $B(t)$ is integrated with respect to z it effectively multiplies by a unit of length (the height), leaving the apparent units of g/cm^2. To arrive at the desired units of grams, recall that for the one-dimensional biofilm the assumption is made that the length and width of the biofilm are unitary in length (so that the magnitude of the height of the biofilm is the same as the magnitude of the volume). Multiplying by the unitary length and width of the biofilm will yield the total mass of bacteria for a given height in grams as desired. For the interest of the reader, the number of bacteria or colony forming units (CFU) may be a better measure of its amount in the biofilm. Thus, the total number of living bacteria in the biofilm at a given height is calculated as follows:

$$Bacteria_{total}(HH[i]) = \frac{fB(HH1[i], XX[i], HH1[i])}{B^*}, \tag{3.14}$$

where i is an iterate between 1 and $mm+1$, and the value of B^* is the average mass of one bacterium. Again to elucidate, consider the unit analysis: recall fB has units of grams. Dividing the total mass of living bacteria in the biofilm, fB, by the average mass per one bacteria will yield the number of bacteria from the base of the biofilm to the desired location in the vertical axis.

Similar to (3.13), functions can be defined for total mass of dead bacteria, persister bacteria and EPS called fB_i, fB_p, and fE respectively. From this, total numbers of dead bacteria and persister bacteria can be calculated in the same way as in (3.14). In the case of EPS, the function fE explaining the mass of EPS at a given location is the best way of explaining the amount present in the biofilm since EPS is not discrete; thus there is no need to define an equation similar to (3.14) for EPS.

After the calculations above, calculate the total number of bacteria for every step i by defining a two dimensional array, $BacteriaBeforeMed$. The first dimension of the array, indicating time in days correlating to total number of bacteria, is found by iterating m from 1 to $mm+1$ and calculating

$$BacteriaBeforeMed[m,1] = \frac{(m-1)*tmeddays}{mm},$$

where $tmeddays$ is the time in days that the antimicrobial is applied. The second dimension of the array, indicating the total number of bacteria, is found by iterating m from 1 to $mm+1$, and calculating

$$BacteriaBeforeMed[m,2] = \frac{fB(HH1[m], XX[m], HH1[m])}{B^*},$$

as indicated in (3.14).

Before finishing with part one of this solution, define the variables:

$$B_{final} = \frac{fB(HH1[mm+1], XX[mm+1], HH1[mm+1])}{HH1[mm+1]}, \tag{3.15}$$

$$B_{i_{final}} = \frac{fB_i(HH1[mm+1], XX[mm+1], HH1[mm+1])}{HH1[mm+1]}, \tag{3.16}$$

$$B_{p_{final}} = \frac{fB_p(HH1[mm+1], XX[mm+1], HH1[mm+1])}{HH1[mm+1]}, \tag{3.17}$$

and

$$E_{final} = \frac{fE(HH1[mm+1], XX[mm+1], HH1[mm+1])}{HH1[mm+1]}, \tag{3.18}$$

which are the final concentrations of living bacteria, dead bacteria, persister bacteria, and EPS respectively for the period of time where antimicrobial is not present. Recall that since this is a one dimensional model, the length and width are assumed to be unitary in length. Thus the magnitude of the height is equal to the magnitude of the volume. That is, $Volume_{biofilm} = 1\,cm \times 1\ cm \times$ (height of biofilm in centimeters). The values B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, and E_{final} calculated here will be used as initial conditions in the second part of the solution where antimicrobial is present.

Before closing this part of the solution, save B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, and E_{final}, H_{final}, t_{med}, and t_{final}.

Part Two: Growth in the Presence of Antimicrobial

This part will be similar to part one, except that the biofilm has already grown, and the antimicrobial will now be solved for in detail. Start the solution procedure by

loading the values for B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, E_{final}, H_{final}, t_{med} and t_{final} from part one of the solution.

For this part, again begin by putting equations (2.30), (2.31), and (2.32) into matrix form. Find the eigenvectors and eigenvalues as before. Place eigenvectors into a matrix called L and define $M = L^{-1}$, as in the previous part. Similarly set up a matrix of initial masses of living bacteria, dead bacteria, and persister bacteria, calling this Q. Compute $P = M * Q$ and find the constants of integration, c_1, c_2, and c_3 as they are the elements of the P matrix as shown in part one of this subsection.

Next, define the constants shown in table A.3. Notice that C_{Source} will change depending on the amount of antimicrobial required on the surface of the biofilm for the particular solution sought. Also, $\kappa \neq 0$, as the antimicrobial is now able to kill the bacteria in the biofilm in this stage of the solution. Finally, the initial masses B_0, B_{p_0}, B_{i_0}, and E_0 are set equal to the loaded values from part one of this solution: B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, and E_{final} respectively.

The solution for $B(t)$, $B_i(t)$, and $B_p(t)$ are found in the same way as above, as is the equation for $E(t)$. In the exact way as part one, solve the differential equation regarding nutrient in the biofilm in equation (3.4) subject to boundary conditions (3.5) and (3.6).

In this part of the solution, the equation for the concentration of antimicrobial is vital, as the antimicrobial is empowered by the presence of a nonzero term κ. To solve for the antimicrobial, the system of ODEs is solved which is a simplification of equation (2.35). For this solution, the two equations in the system are identical,

since the antimicrobial is being delivered on the surface of the biofilm, and constant concentration is desired. The system is divided into two parts, antimicrobial towards the surface of the biofilm called $CS(z)$, and antimicrobial towards the bottom of the biofilm called $CB(z)$. Solve the system as follows:

$$\begin{aligned} CS(z)_{zz} - \frac{k_C}{\phi^2 \bar{D}_C} CS(z) &= 0, \\ CB(z)_{zz} - \frac{k_C}{\phi^2 \bar{D}_C} CB(z) &= 0, \end{aligned} \tag{3.19}$$

subject to the boundary conditions

$$CB(0)_z = 0 \tag{3.20}$$

showing the change in concentration of antimicrobial at the bottom of the biofilm is 0. At the biofilm surface,

$$-\phi^2 \bar{D}_C CS(d)_z = k_{HC} \left(CS(d) - C_{Source} \right), \tag{3.21}$$

which provides a source for the antimicrobial at the height d which will later be replaced by the actual height of the biofilm once calculated. Also,

$$CS(z_0)_z - CB(z_0)_z = -k3_C, \tag{3.22}$$

which is the jump condition for the system around the location of the nanosphere z_0 (if there was a nanosphere present in this part of the solution, here $k3_C = 0$ since constant concentration is considered) and

$$CB(z_0) = CS(z_0), \tag{3.23}$$

which constrains the two equations to be continuous at the point z_0. $C(z)$ is then defined as the piecewise defined function:

$$C(z) = \left\{ \begin{array}{ll} CB(z), & z < z_0 \\ \\ CS(z), & z \geq z_0 \end{array} \right\}. \tag{3.24}$$

The term z_0 is used so that it may be possible to use this solution in the second solution type with the nanosphere antimicrobial delivery, where the location z_0 would be the place where the nanosphere would degrade. In this part of the solution, z_0 is irrelevant, and $CB(z) = CS(z)$ for all z as indicated by the choice of $k3_C = 0$ and equations (3.19). Now solving (3.19) for the given condition that no antimicrobial is lost from the system (i.e. $k_C = 0$) results in the simple solution that $C(z) = C_{Source}$. Consequently, this case considers a constant amount of antimicrobial within the biofilm that is equal to the amount that is placed on the surface of the biofilm under the constraint that nothing leaves the biofilm (deemed the 'no flux' condition).

Now $S(z)$ and $C(z)$ can replace temporary variables SS and CC in the equations for $B(t)$, $B_i(t)$, $B_p(t)$, and $E(t)$ as in part one so that B, B_i, B_p and E now are functions of z. Again, replacement is done at this stage due to the faster time scale with which the particulate species diffuse compared to the other biofilm processes. After substitution, solve for the advective velocity w using equation (3.7).

Now the height can be computed for the period of time where antimicrobial is present. As before, define a value for Δt which is the time-step, and a value for mm which is the partition step size. For the second part of the solution where

antimicrobial is present, $mm = \dfrac{t_{final} * 24 * 3600 - t_{med}}{\Delta t}$, since t_{final} (which must be converted to seconds for calculation purposes) represents the final time in days of the desired examination period and t_{med} is already in units of seconds. Similar to part one, configure two arrays, one for the values that exist in the domain called XX (here, representing the time starting when antimicrobial is administered and all time thereafter in days), and another for those values in the range representing height, this time calling it $HH2$ so that each array contains elements from 1 to mm+1. Initialize the $HH2$ array by defining $HH2[1]$ to the value of the height that is the final height value from part one. That is,

$$HH2[1] = H_{final}. \tag{3.25}$$

To find the rest of the values for H, iterate a variable i from 1 to mm, and define

$$HH2[i+1] = HH2[i] + w(HH2[i], i * \Delta t, HH2[i]) * \Delta t. \tag{3.26}$$

This provides the height for the biofilm at varying times from t_{med} to t_{final}.

As before, set up a two dimensional array called $HeightAfterMed$ to contain the values of the height of the biofilm after antimicrobial is applied and to contain corresponding times using the scheme that follows. Compute the first dimension of the array representing time values for the period between $t = t_{med}$ and $t = t_{final}$ by iterating m from 1 to $mm + 1$ and calculating:

$$HeightAfterMed[m, 1] = \frac{(t_{final} - tmeddays) * (m-1)}{mm} + tmeddays. \tag{3.27}$$

Likewise, compute the elements for the second dimension of the array representing height values by iterating m from 1 to $mm + 1$ and storing as:

$$HeightAfterMed[m, 2] = HH2[m]. \tag{3.28}$$

Concatenating the arrays $HH1$ from part 1 and $HH2$ from part 2 and graphing them with their respective XX values will produce a graph showing the height of the biofilm for the entire period from $t = 0$ to $t = t_{final}$. In the graph, the time $t = t_{med}$ will represent the time when antimicrobial began being applied to the surface of the biofilm.

The number of biofilm components with respect to time are calculated just as in part one, using a setup similar to equation (3.14) replacing HH1 with HH2 for the components B, B_i, B_p and E thus producing functions fB, fB_i, fB_p, and fE. To determine the number of bacteria configure a two-dimensional array called $BacteriaAfterMed$. Let the first dimension represent time in the period of $t \in [t_{med}, t_{final}]$ and calculate the first dimension of the array by iterating m from 1 to mm+1 and computing:

$$BacteriaAfterMed[m, 1] = \frac{(t_{final} - tmeddays) * (m - 1)}{mm} + tmeddays. \tag{3.29}$$

Calculate the second dimension of the array, representing the number of bacteria in the biofilm while antimicrobial is present by computing:

$$BacteriaAfterMed[m, 2] = \frac{fB(HH2[m], XX[m], HH2[m])}{B^*}. \tag{3.30}$$

Concatenating the arrays $BacteriaBeforeMed$ and $BacteriaAfterMed$ and graphing on a two dimensional graph shows the number of bacteria that exist in the biofilm

with respect to time during the period from $t = 0$ to $t = t_{final}$. Note that $t = tmed$ represents the time that the antimicrobial is applied to the surface of the biofilm. Similarly, calculate the number of any other biofilm component desired.

3.2.2 Antimicrobial Delivered Through Nanospheres

This section describes the solution procedure when antimicrobial is not constant and is delivered via nanospheres to the biofilm. Like the first subsection of this chapter, this solution is broken down into two parts: part one where antimicrobial is not present during the period of time from $t = 0$ to $t = t_{med}$ and part two where antimicrobial begins to be delivered by nanospheres during the period of time $t = t_{med}$ to $t = t_{final}$. The solution procedure here will be identical to the first subsection's solution procedure, except the way that the antimicrobial equation is solved. The nanosphere solution works by allowing the nanospheres to be placed at the height z_0 and with diffusion from this location. Similar to the previous case, it is assumed that the antimicrobial will diffuse through a height d that is not yet prescribed and will act as a placeholder for the height of the biofilm once the height equation is solved. Again, d becomes equivalent to the changing height of the biofilm once quantities fB, fB_i, fB_p, and fE are defined later in the solution, and is thus non-constant.

Part One: Growth Before Antimicrobial is Present

The technique for this solution is identical to that explained in the subsection for antimicrobial applied at surface. Naturally, in both cases for antimicrobial delivery, the period of time when antimicrobial is absent should have identical biofilm growth.

Part Two: Growth After Antimicrobial is Delivered Through Nanospheres

Just as in part two of the first solution technique, first start by loading the values for B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, E_{final}, H_{final}, t_{med}, t_{final} from part one of the solution. Put equations (2.30), (2.31), and (2.32) into matrix form as before. Find the eigenvectors and eigenvalues of this matrix. Place eigenvectors into a matrix called L and define $M = L^{-1}$, as done previously. Similarly set up a matrix of initial masses of living bacteria, dead bacteria, and persister bacteria, calling this Q. Compute $P = M * Q$ and find the constants of integration, c_1, c_2, and c_3 as they are the elements of the P matrix as shown in part one of the previous subsection.

Next, define the constants shown in table A.4. Notice that k, being the rate with which the nanosphere degrades, is now vital to the solution. As expected, $\kappa \neq 0$, as the antimicrobial is now able to kill the biofilm in this stage of the solution. Note that κ is dependent on the quantity $m_{spheres}$ which is the number of nanospheres present. That is, with more nanospheres present, more antimicrobial will be present. As κ is multiplied by the amount of antimicrobial C in each governing equation for the model, the dependence of κ on the number of nanospheres implicitly makes the amount of antimicrobial dependent on the number of nanospheres, as desired. Also, the location of the nanosphere z_0, though not utilized in this particular solution, can play a key role in this part of the solution. In the solution presented here, it is assumed that the diffusion of the antimicrobial is very fast (assumed infinite diffusion rate) and thus the location of the nanosphere is unimportant. The variable $k_{HC} = 0$, effectively disabling the application of antimicrobial on the surface so that

all antimicrobial present is delivered by way of nanosphere. The variable $C_0 \neq 0$ since the concentration of antimicrobial inside the nanosphere is now greater than zero. Finally, as before, the initial masses B_0, B_{p0}, B_{i0}, and E_0 are set equal to the loaded values from part one of the solution presented in subsection 3.2.1: B_{final}, $B_{i_{final}}$, $B_{p_{final}}$, and E_{final}, respectively.

The solution for $B(t)$, $B_i(t)$, and $B_p(t)$ and $E(t)$ are found in the same way as in part two of subsection 3.2.1. Also, just as in 3.2.1, solve the differential equation regarding nutrient in the biofilm in equation (3.4) subject to boundary conditions (3.5) and (3.6).

The solution for the antimicrobial performed next differs from those done previously. Here, consider two limiting cases for the diffusion of antimicrobial in equation (2.35) derived from [26]. First, consider when no diffusion from the nanosphere containing antimicrobial occurs: that is, $D_C = 0$. In this case, (2.35) (changing the variable name from R to R_C as used in the model) simplifies to,

$$\phi C_t = \delta(z - z_0) \left\{ \begin{array}{ll} 4\pi {R_C}^2 k \left[1 - \frac{kt}{C_0 R_C}\right]^2, & 0 \leq t \leq \frac{C_0 R_C}{k} \\ 0, & t > \frac{C_0 R_C}{k} \end{array} \right\}. \tag{3.31}$$

Integrating both sides of equation (3.31) with respect to time and recalling that $C(0) = 0$ and $C\left(\frac{C_0 R_C}{k}\right) = \frac{4}{3}\pi {R_C}^3 C_0$ yields

$$\phi C = \delta(z - z_0) \left\{ \begin{array}{ll} \frac{4}{3}\pi {R_C}^3 C_0 \left[1 - \left(1 - \frac{kt}{C_0 R_C}\right)^3\right], & 0 \leq t \leq \frac{C_0 R_C}{k} \\ \frac{4}{3}\pi {R_C}^3 C_0, & t > \frac{C_0 R_C}{k} \end{array} \right\}. \tag{3.32}$$

Dividing both sides of equation (3.32) by ϕ shows that for the no diffusion case, antimicrobial concentration is given by,

$$C(z,t) = \frac{\delta(z-z_0)}{\phi} \left\{ \begin{array}{cc} \frac{4}{3}\pi {R_C}^3 C_0 \left[1 - \left(1 - \frac{kt}{C_0 R_C}\right)^3\right], & 0 \leq t \leq \frac{C_0 R_C}{k} \\ \frac{4}{3}\pi {R_C}^3 C_0, & t > \frac{C_0 R_C}{k} \end{array} \right\}. \quad (3.33)$$

This solution represents a point source for antimicrobial at the location z_0 that does not diffuse from the location z_0.

In the solution for nanosphere delivery presented in this work, the second limiting case of infinite diffusion ($D_C \to \infty$) is studied in detail. Consider integrating each term in (2.35) (again, changing the variable name from R to R_C as used in the model) from $z = 0$ to $z = d$, where d represents the height of the biofilm. This yields,

$$\phi \int_0^d C_t \mathrm{d}z - \phi D_C C_z \Big|_0^d = \left\{ \begin{array}{cc} 4\pi {R_C}^2 k \left[1 - \frac{kt}{C_0 R_C}\right]^2, & 0 \leq t \leq \frac{C_0 R_C}{k} \\ 0, & t > \frac{C_0 R_C}{k} \end{array} \right\}. \quad (3.34)$$

The right side of (2.35) was simplified to the right side of (3.34) by recalling that z_0 is some point interior to the interval $[0, d]$ and thus the integral of the delta function will equal unity. In this case with infinite diffusion, the change in concentration of antimicrobial is not expected to be spatially dependent. Thus, in the first term of (3.34), C_t can be removed from the integral. The second term of (3.34), when evaluated with the 'no flux' boundary conditions, equals zero. Thus (3.34) is equivalent to

$$\phi C_t d = \left\{ \begin{array}{cc} 4\pi {R_C}^2 k \left[1 - \frac{kt}{C_0 R_C}\right]^2, & 0 \leq t \leq \frac{C_0 R_C}{k} \\ 0, & t > \frac{C_0 R_C}{k} \end{array} \right\}. \quad (3.35)$$

Integrating both sides of equation (3.35) with respect to time produces the following equation governing the amount of antimicrobial present in the biofilm of height d at time t for the case of infinite diffusion:

$$C(z,t,d) = \left\{ \begin{array}{ll} \left(\frac{4}{3}\pi {R_C}^3 C_0\right) \frac{1-\left(1-\frac{kt}{R_C C_0}\right)^3}{\phi d}, & 0 \leq t \leq \frac{C_0 R_C}{k} \\ \frac{\left(\frac{4}{3}\pi {R_C}^3 C_0\right)}{\phi d}, & t > \frac{C_0 R_C}{k} \end{array} \right\}, \tag{3.36}$$

where the integrating constant equals zero due to initial conditions $C(0) = 0$ and since the right hand side of (3.35) evaluated at $t = 0$ equals zero as well. Notice that although the height of the biofilm d does depend on time, it can be removed from the integral due to separation of time scales. That is, the change in the height of the biofilm is much slower than the time-scale for the degradation of a nanosphere, and thus the height d can be treated as a constant. Figure 3.1 shows how the concentration of antimicrobial varies with time using equation (3.36). The values for R_C, C_0, and k are those found in table A.4 in the appendix. For figure 3.1, the height d was chosen to be held constant at .01 cm. As a result, the concentration of antimicrobial continues to increase until day 4 (when all antimicrobial has been released), and then plateaus after day four as there is no way for the antimicrobial to leave the system. Thus in the solution procedure, define $C(z,t,d)$ as seen in equation (3.36). As before, the quantity d is not explicitly known at this stage of the solution, and will act as a place holder for height once the height equation is solved.

After $C(z,t,d)$ is defined, CC and SS can be replaced by $C(z,t,d)$ and $S(z)$ as before into expressions for $B(t)$, $B_i(t)$, $B_p(t)$, and $E(t)$ as in part one of subsection

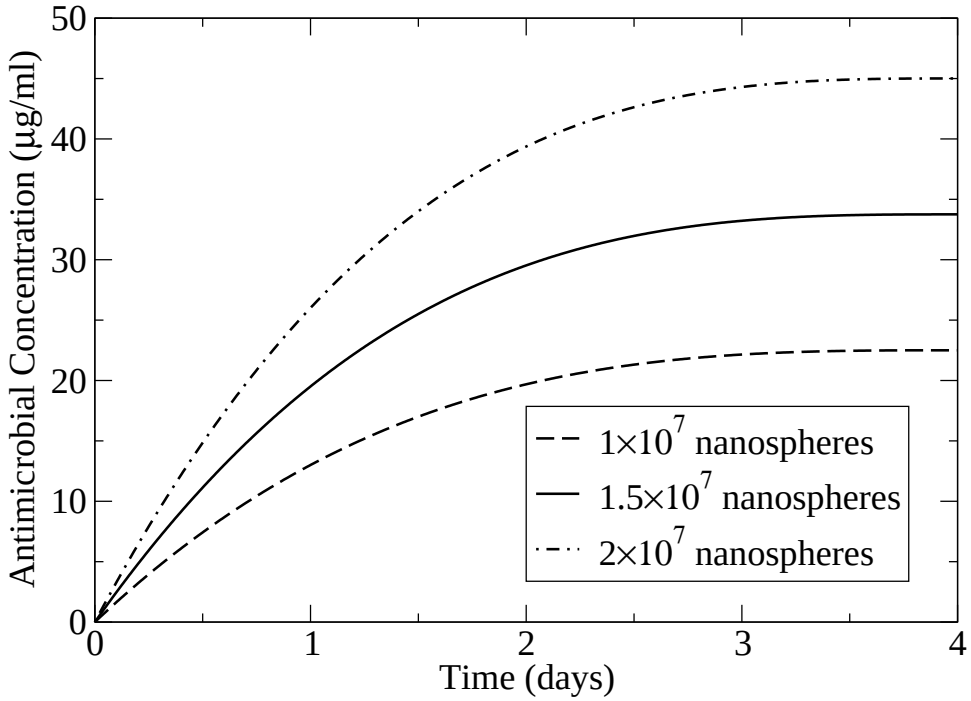

Figure 3.1: Antimicrobial concentrations arising from three quantities of nanospheres using equation (3.36) with values chosen from the appendix in table A.4 so that all antimicrobial is released by day four; note the height of the biofilm, d, is held constant at .01 cm

3.2.1 so that B, B_i, B_p and E now change with respect to z. Solve for the advective velocity w using equation (3.7) and the height can then be computed in the same way as done in part two of subsection 3.2.1, defining mm, Δt, and an array $HH2$. The two dimensional array $HH2$ is calculated and filled using the scheme described in equations (3.25), (3.26), (3.27), and (3.28).

Concatenating the arrays $HH1$ from part 1 and $HH2$ from part 2 of this solution and graphing them with their respective XX values will produce a graph

showing the height of the biofilm for the entire period from $t = 0$ to $t = t_{final}$. Here, in the graph the time $t = t_{med}$ will represent the time when antimicrobial began being delivered via nanospheres.

The number of biofilm components with respect to time are calculated similarly to (3.14) in the previous subsection. As before, the expressions for the particulates in the biofilm are now dependent on the position d, and thus the placement of the nanosphere containing antimicrobial.

To determine the number of bacteria, follow the same scheme as before, defining a two-dimensional array called $BacteriaAfterMed$. Use equations (3.29) and (3.30) to fill the array.

Exactly as in the previous case, concatenating the arrays $BacteriaBeforeMed$ and $BacteriaAfterMed$ and graphing on a two dimensional graph shows the number of bacteria that exist in the biofilm with respect to time during the period from $t = 0$ to $t = t_{final}$. Note that $t = t_{med}$ is the time when antimicrobial begins being delivered through nanospheres.

Finally, to see the concentration of antimicrobial that is delivered, by varying number of nanospheres (by changing the quantity $m_{spheres}$), follow this scheme for filling a two dimensional array $ConcentrationAfterMed$. The first dimension represents time in days and the second dimension represents the amount of antimicrobial present in the biofilm at the given time. This dimension of the array can be filled using the following scheme.

Iterate m from 1 to mm+1 and compute:

$$ConcentrationAfterMed[m,1] = \frac{(tfinal - tmeddays) * (m-1)}{mm} + tmeddays. \tag{3.37}$$

Recall, the expression $C(z,t,d)$ is an expression depending on t and d as unknowns as seen in its definition from equation (3.36). Fill the second dimension of the $ConcentrationAfterMed$ array by iterating m from 1 to mm+1 and computing:

$$ConcentrationAfterMed[m,2] := m_{spheres} * C(HH2[m], XX[m], HH2[m]). \tag{3.38}$$

That is, $XX[m]$ is substituted into the $C(z,t,d)$ expression for t, and $HH2[m]$ is substituted into the $C(z,t,d)$ expression for both z and d. The dependence of concentration of antimicrobial on the number of nanospheres present is explicitly seen in the above equation. In contrast, the presence of nanospheres is recognized in the solution by multiplying the term κ by $m_{spheres}$; since κ does not appear in the definition of the function $C(z,t,d)$, it must be present in (3.38) to assure that the nanosphere dependence is considered when viewing the data produced for graphing. Graphing this array with different selected values for $m_{spheres}$ produces a graph showing the effect of the number of nanospheres on the amount of antimicrobial present in the biofilm at varying times.

CHAPTER IV

RESULTS AND DISCUSSION

In this section the graphs produced through the methods discussed in chapter 3 will be discussed. In the first solution, parts one and two, the biofilm was modelled to have grown for three days in the absence of antimicrobial and for a subsequent four days in the presence of antimicrobial. The number of colony forming units (CFU) was studied versus time.

Two cases were studied under two different delivery methods for antimicrobial. First the case of a low initial persister population of 32 was considered with the initial living bacteria population of 160 with constant concentration of antimicrobial being delivered to the surface. Next, the case of a higher initial persister population of 64 with the initial living bacteria population of 160 was considered for constant concentration of antimicrobial being delivered to the surface as the literature suggests a topical treatment would be effective [19]. In each, to ensure that a proper comparison could be made, the initial height h_0 was chosen so that conservation of volume was maintained. Subsequently, studies were done with the lower persister population to determine the role of the terms k_f and k_R. Finally, the same two cases for low and high initial persister populations were studied with delivery of antimicrobial using nanospheres for the case of infinite diffusion of antimicrobial from the nanosphere.

The graphs presented were calibrated for the low persister case using selected values for κ_g and κ as seen in tables A.1 and A.3. Recall, κ_g represents the ability for living bacteria to transform nutrient into growth, and κ represents the ability of an antimicrobial to cause bacterial death. These values were selected to match data given for an antimicrobial created at The University of Akron with the bacterial strain studied in [33]. Changing the values for κ_g and κ would allow use of this model with other bacterial strains and antimicrobials.

4.1 Low Initial Persister Population with Constant Concentration of Antimicrobial

In this section, the first case of a lower initial persister population is discussed. Initially, there are 160 living bacteria and 32 persister bacteria in the biofilm for this case. As seen in figure 4.1, during the first time period of $0-3$ days, the living bacteria experience exponential growth as is expected from the definition of $B(t)$ in equation (3.1). Since $\kappa = 0$ during this period of time, antimicrobial is effectively nullified in each of the governing equations, and thus the bacteria are able to grow well. Much like true bacteria, there is a slow start-up for the growth of the modelled bacteria in the biofilm, commonly referred to as the lag phase, which physically represents a time wherein the bacteria are acclimating to their environment, as seen during the time period of $0 - 2$ days. After this initial lag phase of growth, the bacteria enter into a rapid growth phase with a steep positive slope on the population graph commonly referred to as the log phase of growth, seen during the time period of $2 - 3$ days [2]. After day three, the term $\kappa = 1 \times 10^6$, thus allowing the antimicrobial to

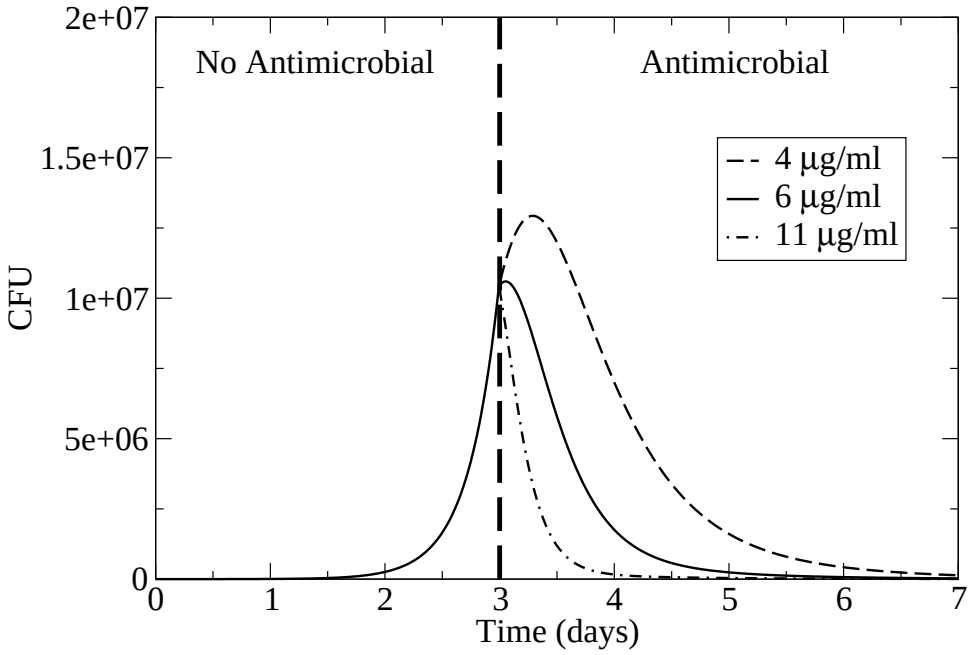

Figure 4.1: Living bacteria growth and decay in biofilm conditions where antimicrobial is constantly applied to surface from day three until day seven with low initial quantity of persister bacteria

be activated. As the quantity C_{Source} is changed in the solution, the three different graphs can be produced as seen on the right side of figure 4.1. For clarification, $C_{Source} = 4 \times 10^{-6} \frac{g}{cm^3}$, $C_{Source} = 6 \times 10^{-6} \frac{g}{cm^3}$, and $C_{Source} = 11 \times 10^{-6} \frac{g}{cm^3}$ in table A.3 correlate to antimicrobial concentrations of 4 μg/ml, 6 μg/ml, and 11 μg/ml, respectively. From the figure, it is apparent that 4 μg/ml of the antimicrobial is insufficient to stop the growth of the modelled strain of bacteria immediately. However, as $B(t)$ is composed of the sum of three exponentials as explained in chapter 3, at approximately 3.5 days the decaying exponential(s) dominated the increasing exponential

and started the decrease of living bacteria within the biofilm. This is in accordance with biological phenomena viewed in the laboratory. Though an antimicrobial may be insufficient to immediately stop the growth of bacteria, it may shift bacteria from their log phase of growth into what is known as a stationary phase of growth wherein the bacteria do not have sufficient volume or nutrients to keep growing at the rate they once were. After time, the bacteria will leave the stationary phase and enter the death phase. Here, as seen in figure 4.1, the bacteria enter death phase within the biofilm at approximately 3.5 days. The introduction of antimicrobial into the system causes the living bacteria to die. In addition, the conservation term α, which mandates that volume be conserved within the biofilm contributes to living bacteria's death. As stated earlier in the model description, the conservation term α is the model's mechanism for keeping the bacteria from growing without spatial constraint. This can be physically interpreted as the living bacteria having insufficient access to resources that contribute to growth due to the large presence of other biofilm particulates. The dosage level of 6 μg/ml is close to what is known as the minimum inhibitory concentration (MIC) or the minimum concentration of antimicrobial required to prevent bacteria from growing once administered [34]. In contrast to the dosage level of 4 μg/ml, 6 μg/ml immediately causes the bacteria within the biofilm to enter their death phase, and quickly kills the living bacteria within the biofilm. The dosage amount of 11 μg/ml is highly effective at killing the bacteria in the biofilm, but is much higher than the MIC as the decaying exponential exhibits. This dosage level, known as the minimum bactericidal concentration (MBC), decreases the population

of living bacteria by 99% within one day [34]. Within the field of medicine, one must balance the threshold for what is above the MIC, but below the toxic level for the recipient of the drug [35]. In some instances, the toxic levels for antimicrobials can be high, as has been seen in preliminary studies from the Center of Silver Therapeutic Research (CSTR) at The University of Akron [36]. These antimicrobial options have been shown to be highly effective at killing bacteria, yet only be toxic to humans at very high concentrations.

To the interest of the biologist, living bacteria populations one day after after antimicrobial is applied to biofilm surface for varying concentrations of antimicrobial is shown in figure 4.2. This figure presents the same information seen in figure 4.1 in an alternative format. Note also, that without the presence of antimicrobial, the living bacteria continue to grow well as indicated by the first bar labeled 'media' in figure 4.2.

It is of interest to the modeller to better understand the nature of persister bacteria as a mechanism of resistance for living bacteria within a biofilm. First an initial persister population of 32 bacteria was considered. Figure 4.3 depicts that the persister bacteria populations have similar profiles to the living bacteria as seen in figure 4.1. That is, the maximum size of the bacterial populations is inversely proportional to the concentration of antimicrobial administered, as expected. Interestingly, there is a delay between the time that antimicrobial is first administered at day 3 and the decrease of the persister populations for each of the concentrations of antimicrobial. Even the highest concentration of 11 μg/ml is not able to kill the

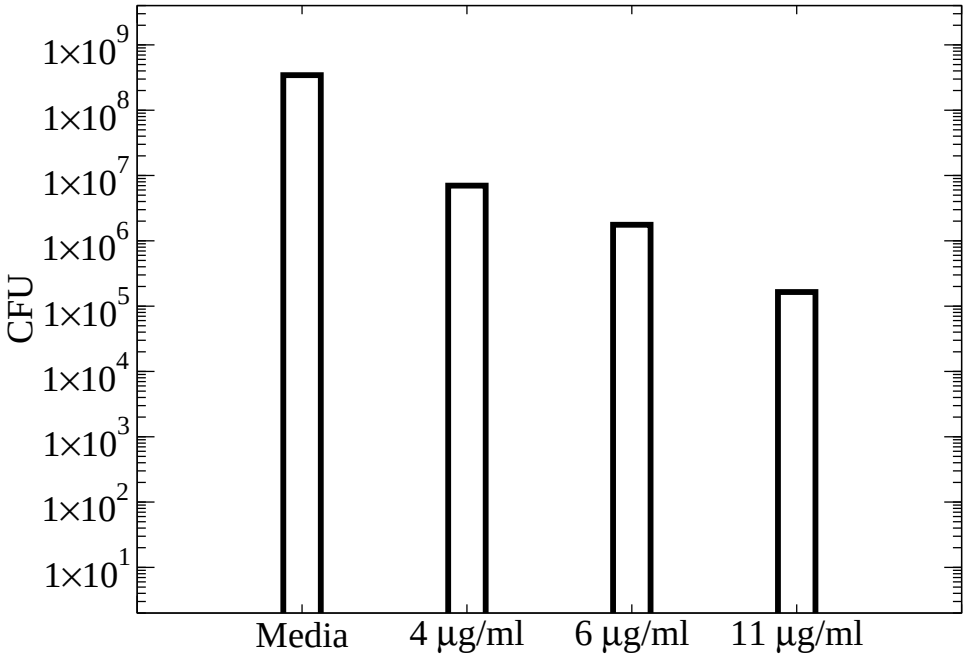

Figure 4.2: Living bacteria population sizes one day after antimicrobial is applied to surface of 3-day biofilm for varying concentrations of antimicrobial; the term 'media' indicates no antimicrobial was applied and acts as the control

persister bacteria immediately. This is in accordance with the theory that persister bacteria are not susceptible to antimicrobial and must first revert to living bacteria before antimicrobial may contribute to death. Moreover, the exponential growth of the persister bacteria is in accordance with the literature regarding persisters growing in planktonic environments [2]. In this manner, the biofilm can stave off the threat of the antimicrobial until it is no longer present, and then repopulate the biofilm.

The height of the biofilm was also studied in this case, as seen in figure 4.4. For the dosage levels that are able to kill the bacteria quickly (6μg/ml and 11μg/ml),

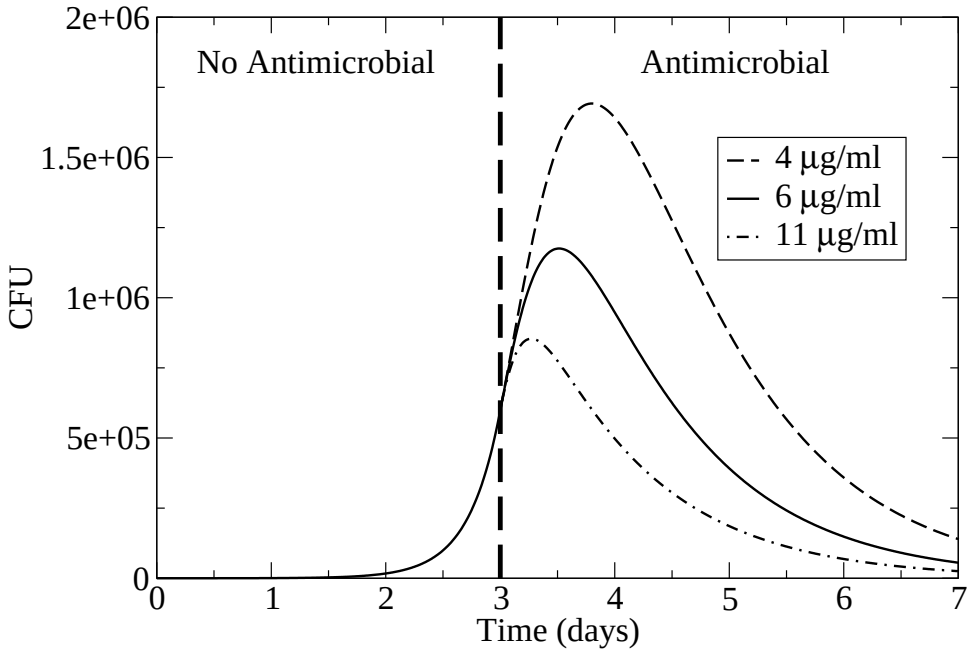

Figure 4.3: Persister bacteria growth and decay in biofilm conditions where antimicrobial is constantly applied to surface from day three until day seven with low initial quantity of persister bacteria

the height of the biofilm is kept to levels similar to those found in the literature [11]. With the dosage level of 4μg/ml, at which bacterial death is not immediately noticed upon introduction of the antimicrobial, the height of the biofilm grows to values larger than those found in the literature. One weakness of this 1-D model, affecting its accuracy, is the fourth modification made to the original 2-D model discussed in chapter 2 section 2.2 to remove detachment terms which enables the biofilm height to realistically degrade over time. Thus it is expected that given enough time, and with large enough bacterial populations, the height of the biofilm will reach greater

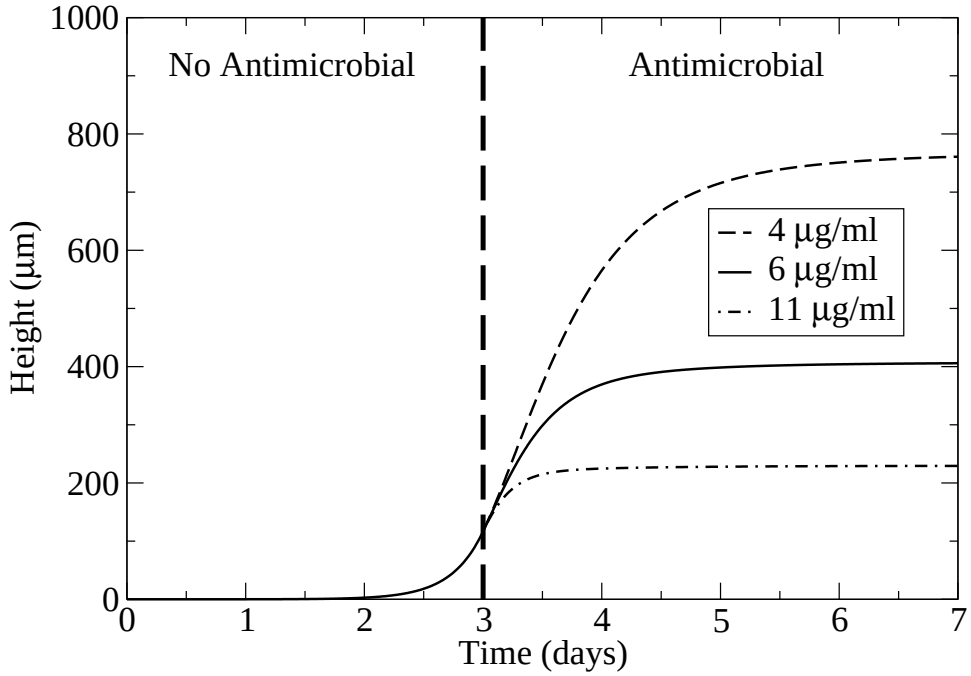

Figure 4.4: Height of biofilm where antimicrobial is constantly applied to surface from day three until day seven with low initial quantity of persister bacteria

than realistic levels. Nonetheless, for dosage levels at or below the MIC, the height of the biofilm falls within standard literature values [11] for the modelled case of lower initial persister populations.

4.2 High Initial Persister Population with Constant Concentration of Antimicrobial

In this section, the second case of higher initial persister population is discussed. Initially, there are 160 living bacteria and 64 persister bacteria in the biofilm. Figure 4.5 depicting higher initial persister populations shows nearly identical shape to that

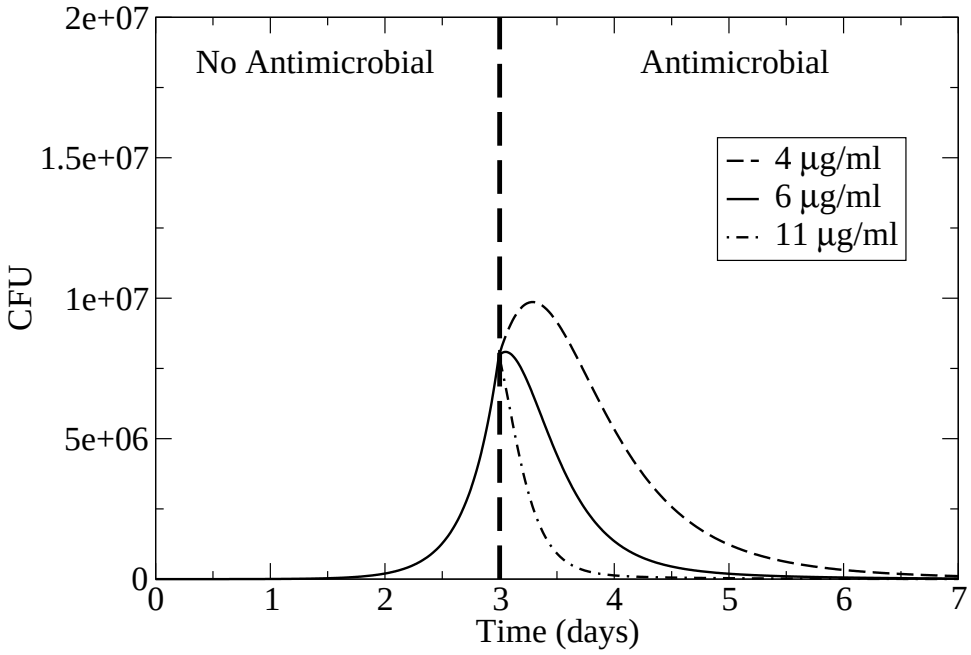

Figure 4.5: Living bacteria growth and decay in biofilm conditions where antimicrobial is constantly applied to surface from day three until day seven with high initial quantity of persister bacteria

seen previously for the lower initial persister populations in figure 4.1. The only difference is that the bacteria population graph profile for higher initial persister populations is a vertical shrink of the bacteria population graph profile for lower initial persister population (taking note of the different vertical axes). Thus the higher initial persister population actually keeps the living bacteria population from growing as high as the lower initial persister population allowed. Mathematically, at time $t = 0$, the conservation term α, seen in equation (2.30), is made larger by the presence of a higher initial persister population. Thus initially the living bacteria with

higher initial persister populations will grow at a slower rate than those living bacteria with lower initial persister populations. This slow start-up for growth contributes to a lower maximum living population for the higher initial persister population case. Physically, this represents that a biofilm that has a greater proportion of its population in the dormant persister state would not be as likely to grow when subject to the constraint that ϕ is constant. As discussed previously, when bacteria are in their first phase of growth, the lag phase, they are acclimating to their environment and preparing for the log phase of growth. In this case the result demonstrates that the initial acclimation phase of the living bacteria is essential to their future growth. The living population does not grow as readily due to the higher number of initial persister cells in the biofilm. Moreover, the result suggests that in order for the biofilm to grow the most living bacteria, the optimal initial population of persisters should be low.

The persister populations results for the case of higher initial persister populations is considered in figure 4.6. Like the living bacteria, the growth profiles for the persister populations in both the case for lower initial persister population and higher initial persister population are the same aside from a vertical shrink. That is, the persister population graph profile for the case of higher initial persister populations is a vertical shrink of the persister population graph profile for the case of lower initial persister populations. As seen in the governing equation for the persister bacteria growth in chapter 2 in equation (2.32), only two terms govern the growth or decay of the persister population: the living bacteria population and the persister

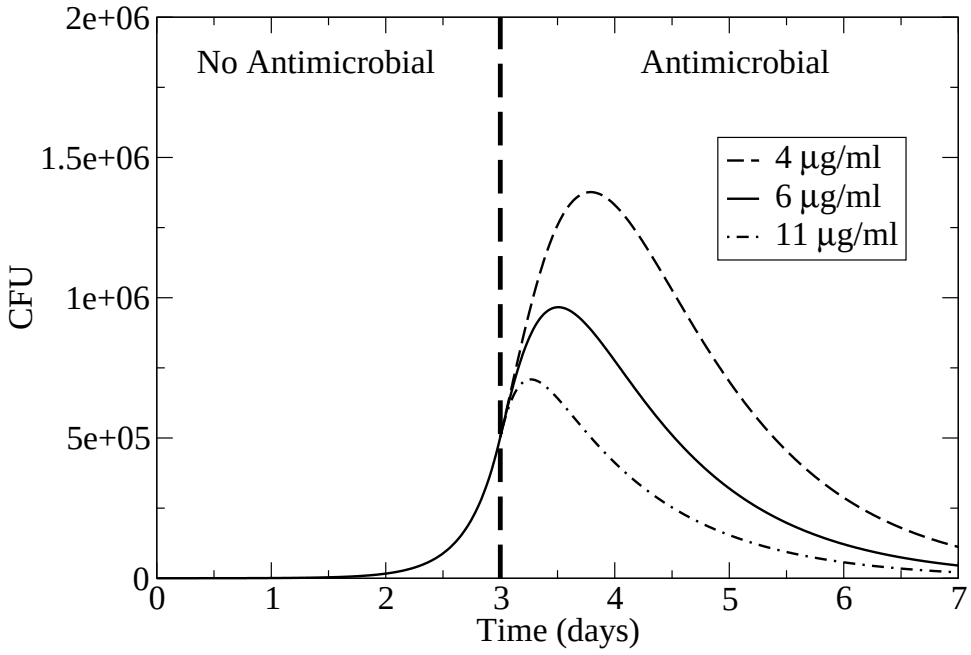

Figure 4.6: Persister bacteria growth and decay in biofilm conditions where antimicrobial is constantly applied to surface from day three until day seven with high initial quantity of persister bacteria

bacteria population. Higher living bacteria populations increase the persister population's rate of growth. Conversely, a higher persister population actually decreases the persisters' rate of growth. Thus as seen in figure 4.6, the initial higher persister populations contribute to a slower rate of growth for the persister bacteria. Moreover, as discussed in the previous paragraph, the lower living bacteria population resulting from higher initial persister population would lead to a slower rate of growth for the persister bacteria as well. Again, the results of the two cases for the initial persister

populations suggest that in order to grow higher bacterial populations within the biofilm, a lower persister population should be present initially.

The height of the biofilm was also considered for this case of higher initial persister populations as can be seen in figure 4.7. As expected by the lower overall populations of living and persister bacteria, the height did not grow as large as it did for the cases of lower initial persister populations. As seen in the previous case, the dosage level of 4μg/ml, which is below the MIC, allows the biofilm height to grow to approximately 600μm which is more than typically expected for biofilms to grow

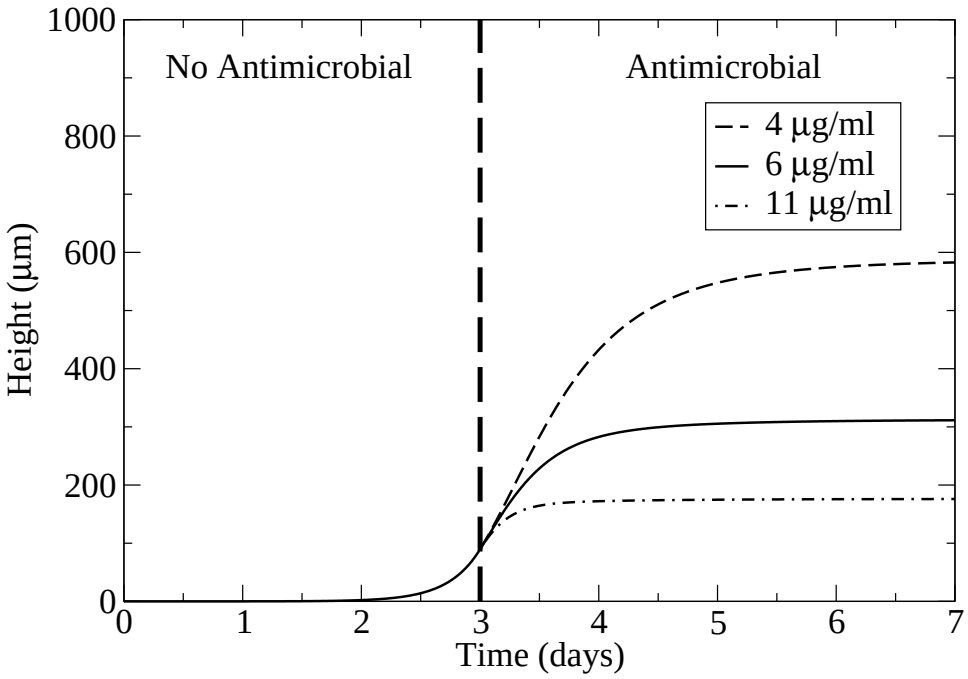

Figure 4.7: Height of biofilm where antimicrobial is constantly applied to surface from day three until day seven with high initial quantity of persister bacteria

except in rare cases such as waste-water treatment plants where biofilms have been known to grow up to 2000μm [29]. The dosage levels of 6μg/ml and 11μg/ml keep the height within reasonable values for many biofilms [11]. Nonetheless, the height of biofilms is largely determined by the species of bacteria as shown in [31], and the height values produced for dosage levels of 6μg/ml and 11μg/ml seen here may be unreasonable for certain species of biofilm.

4.3 Comparing the Terms k_f and k_R for the Case of a Lower Initial Persister Population

In this section, the results of the parametric study of the terms k_f and k_R will be discussed. Recall, the term k_f refers to the rate at which living bacteria form into persister bacteria, and the term k_R refers to the rate at which persister bacteria revert to living bacteria. Thus, a high value for k_f correlates to a fast rate of formation of persisters from living bacteria. Conversely, a high value for k_R correlates to a fast rate of persisters reverting to living bacteria. The base case for comparison was the dosage level of 6μg/ml applied to the surface of the biofilm for the low initial persister population. As discussed in the previous section, 6μg/ml was the MIC for the modelled antimicrobial. In each of the figures depicting the results of this comparison (figures 4.8, 4.9 and 4.10), the base case is represented with a solid line as noted in the captions. In the figures that follow, five subcases will be described: (1) k_f and k_R equal to the values found in table A.1; (2) k_f held equal to the value found in table A.1 and k_R raised an order of magnitude from the value found in table

A.1; (3) k_f held equal to the value found in table A.1 and k_R lowered an order of magnitude from the value found in table A.1; (4) k_R held equal to the value found in table A.1 and k_f raised an order of magnitude from the value found in table A.1; and (5) k_R held equal to the value found in table A.1 and k_f lowered an order of magnitude from the value found in table A.1. These subcases will be referred to by the numbering just stated and correlate to the numbering of the items within the key of each of the figures. In this manner, an understanding of the role of these parameters in the modelling of living and persister bacteria can be ascertained.

Consider that the values for k_f and k_R in table A.1 are such that k_f is approximately two orders of magnitude smaller than k_R for the base case: subcase (1). Figure 4.8 shows that by increasing the disparity between k_f and k_R (such that k_R is still greater than k_f) as in subcases (2) and (5), the living bacteria population reaches a higher maximum value. As discussed previously, the bacteria within the biofilm will grow better when there is more living bacteria than persister bacteria. Since k_R is approximately three orders of magnitude larger than k_f for the subcases (2) and (5), the living bacteria population grows much higher than it does in the conditions of subcase (1). Moreover, subcase (2) has a higher maximum living bacteria population than subcase (5) since increasing the magnitude of k_R increases the growth rate of the living bacteria as seen in the governing equation (2.30). Subcases (3) and (4) show that by decreasing the disparity between the values of k_f and k_R the living bacteria population will not reach as high a value as it did in subcase (1). Living bacteria population growth should be hindered since subcase (3) depicts a low reversion rate

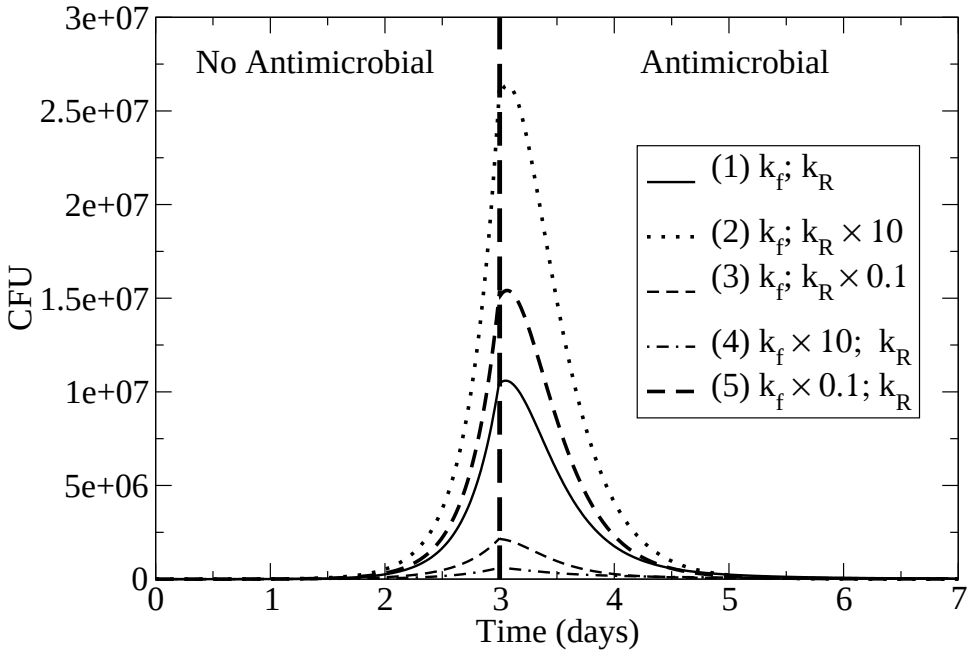

Figure 4.8: Comparison of terms k_f and k_R and their effect on living bacteria populations; the values for the base case k_f and k_R correlate to the data in table A.1 and as noted in the graph were either held constant, increased by an order of magnitude, or decreased by an order of magnitude; a constant antimicrobial concentration of 6μg/ml was applied to surface of biofilm from days three through seven

of persisters to living bacteria and subcase (4) depicts increasing the rate of forming persisters from living bacteria. Since persister bacteria are created from living bacteria (and the converse is also true), an increase in persister bacteria growth should be inversely proportional to an increase in living bacteria growth. In addition, subcase (3) has a higher growth rate for living bacteria than subcase (4) since the magnitude of k_f is smaller in subcase (3) than in subcase (4), in turn forming less persisters from living bacteria. Notice, the magnitude of k_R is smaller in subcase (3) than in

subcase (4). Although equation (2.30) indicates that a higher k_R value would increase the rate of growth of the living bacteria suggesting that living bacteria population size would be hindered, the persister population is smaller than the living bacteria population, and thus the role of k_f dominates over the role of k_R in equation (2.30). These results show that both the disparity between k_f and k_R and the magnitude of these parameter values has an effect on the growth rate of the living bacteria. Interestingly, in all subcases shown in figure 4.8, the MIC is unaffected. As seen previously when comparing the two initial persister populations, the comparative size of the populations of living bacteria and persister bacteria does not change the MIC. This demonstrates that the concentration of antimicrobial dominates the growth rate of living bacteria over the formation of persisters and reversion of persisters to living bacteria in equation (2.30).

Figure 4.9 shows the role of k_f and k_R in the persister population. As explained previously, an increase in persister bacteria growth should be inversely proportional to an increase in living bacteria growth. Thus subcases (2) and (5) that correlate to the two largest living bacteria populations also correlate with the subcases for the two smallest persister populations. This is because the terms k_f and k_R, seen in the governing equation for the persister population growth, equation (2.32), have the opposite effect on persister populations than they do on living bacteria populations. This inverse relationship does not hold for each subcase however. Notice that subcase (3) is the only subcase where persister bacteria grow to a higher value than subcase (1). In addition, the growth prior to day 3.5 for both subcase (1) and sub-

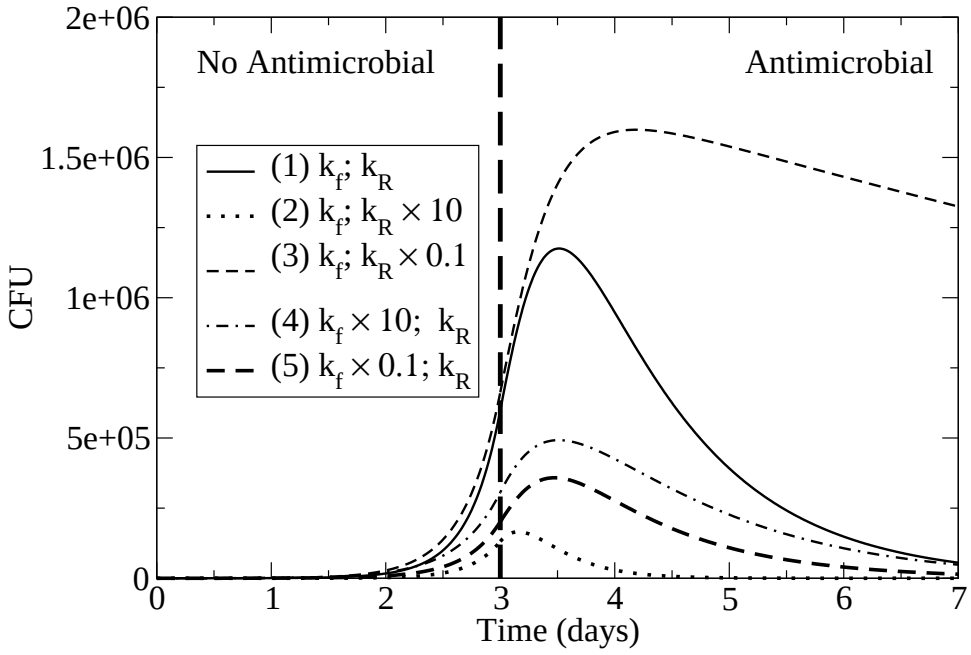

Figure 4.9: Comparison of terms k_f and k_R and their effect on persister bacteria populations; the values for the base case k_f and k_R correlate to the data in table A.1 and as noted in the graph were either held constant, increased by an order of magnitude, or decreased by an order of magnitude; a constant antimicrobial concentration of 6μg/ml was applied to surface of biofilm from days three through seven

case (3) are similar. In both subcase (1) and subcase (3) k_f is smaller in magnitude than k_R; thus k_R tends to dominate equation (2.32) governing persister population growth. As a result, subcase (1) and subcase (3) have similar graphs when persister populations are small. As persister populations grow in size, the role of k_R becomes more important, and the difference in order of magnitude of k_R in subcases (1) and (3) allows subcase (3) to have higher persister population growth. Once subcase (3) reaches its maximum persister population size at approximately day 4, it decreases

slowly and almost linearly. Considering figure 4.8 once more, one can see that at day 4, the population of living bacteria is close to zero for subcase (3). Thus the governing equation for persister bacteria (2.32) is approximately equivalent to

$$B_{p_t} = -k_R B_p. \tag{4.1}$$

Equation (4.1) mandates that the population of persister bacteria be exponentially decaying with rate of decay equal to $-k_R$. Note that since the magnitude of k_R is small, the exponential decay is slow for subcase (3) as seen in figure 4.9. This result shows for certain values of k_f and k_R living bacteria may be practically absent from the biofilm, yet the persister bacteria remain. As suggested in the literature, this is one of the reasons that infections involving biofilms are exceedingly difficult to eradicate.

Now, consider that for subcase (4) persister population growth is less than in subcase (1). Again, the population size of living bacteria plays a key role in the formation of persisters. Looking at figure 4.8, subcase (4) correlates to a maximum living bacteria population size of less than 1 million CFU whereas subcase (1) correlates to a maximum living population size of approximately 10 million CFU. This disparity in the size of living bacteria populations is greater than the disparity in the size of k_f for the two subcases. As a result, referring back to figure 4.9, subcase (1) allows for greater growth of persisters than does subcase (4).

Finally, the effect of the parameters k_f and k_R on the height of the biofilm are the same as the effect on the living bacteria populations. The subcases correlating to

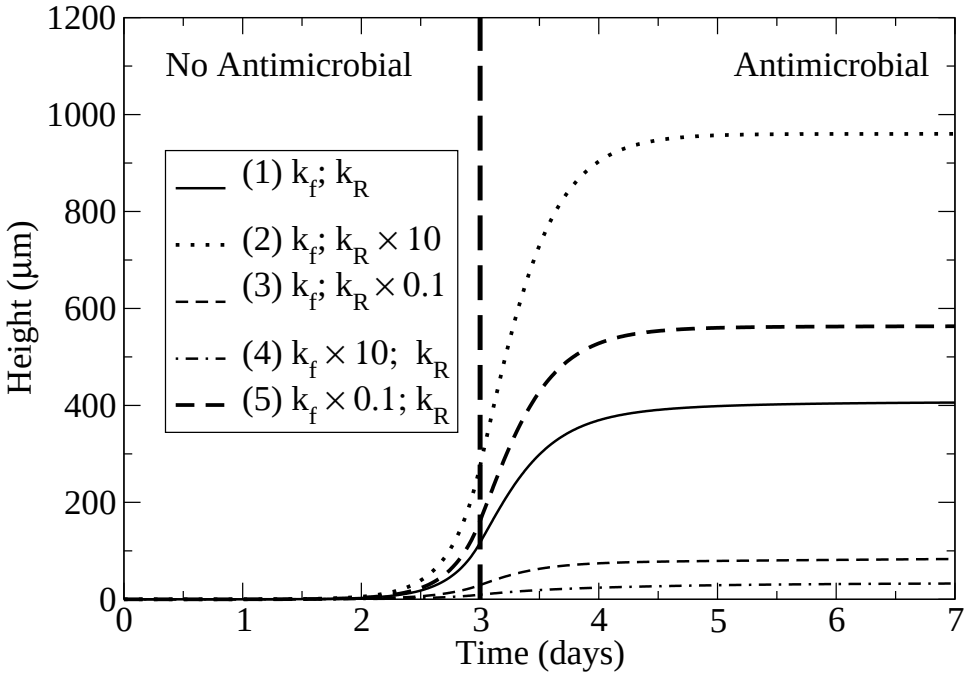

Figure 4.10: Comparison of terms k_f and k_R and their effect on height of biofilm; the values for the base case k_f and k_R correlate to the data in table A.1 and as noted in the graph were either held constant, increased by an order of magnitude, or decreased by an order of magnitude; a constant antimicrobial concentration of 6μg/ml was applied to surface of biofilm from days three through seven

largest living bacteria populations are the exact same subcases that correlate to the greatest biofilm heights. The role of the living bacteria populations in the height of the biofilm is seen in the advective velocity equation (2.34). The larger the advective velocity, the higher the biofilm will grow, since the height of the biofilm is found by integrating the velocity equation as explained in chapter 3. Thus since the living bacteria population is larger than the persister populations in all subcases, it dominates the advective velocity equation. Moreover, the living bacteria also contribute to the

production of EPS as seen in (2.33), and thus has even more of a dominating role in the advective velocity equation and consequently the height of the biofilm.

4.4 Low Initial Persister Population with Antimicrobial Delivered via Nanospheres

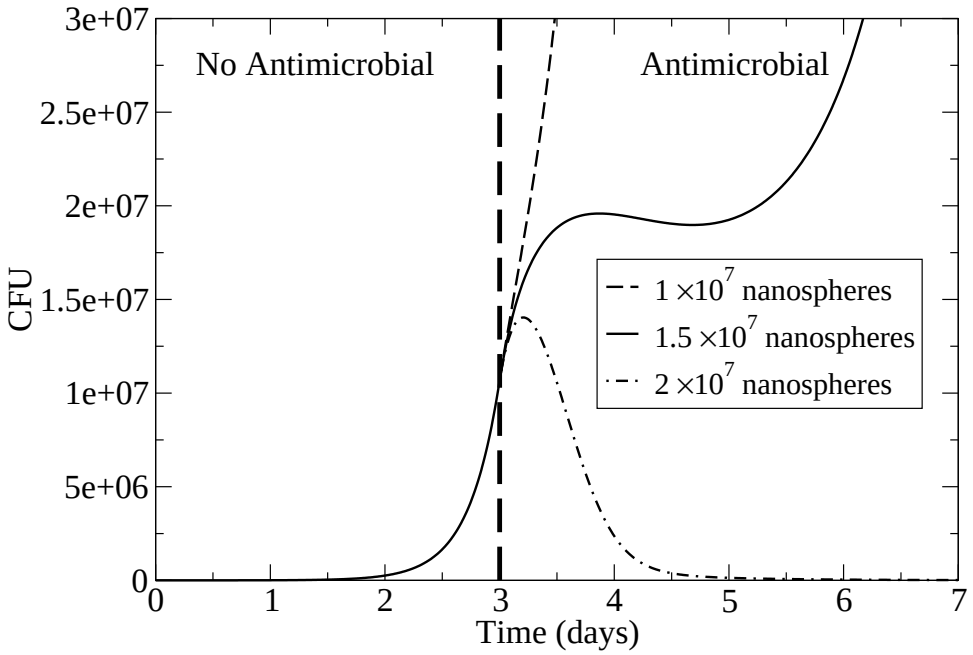

Figure 4.11: Living bacteria growth and decay in biofilm conditions where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with low initial quantity of persister bacteria

In figure 4.11 one curve depicts living bacteria growth for days $0-3$. Subsequently, three curves depict the bacteria population with varying amounts of nanospheres delivered to the biofilm surface at day three. The literature has shown that nanospheres would adequately attach to biofilms and transport inside and it is considered a promis-

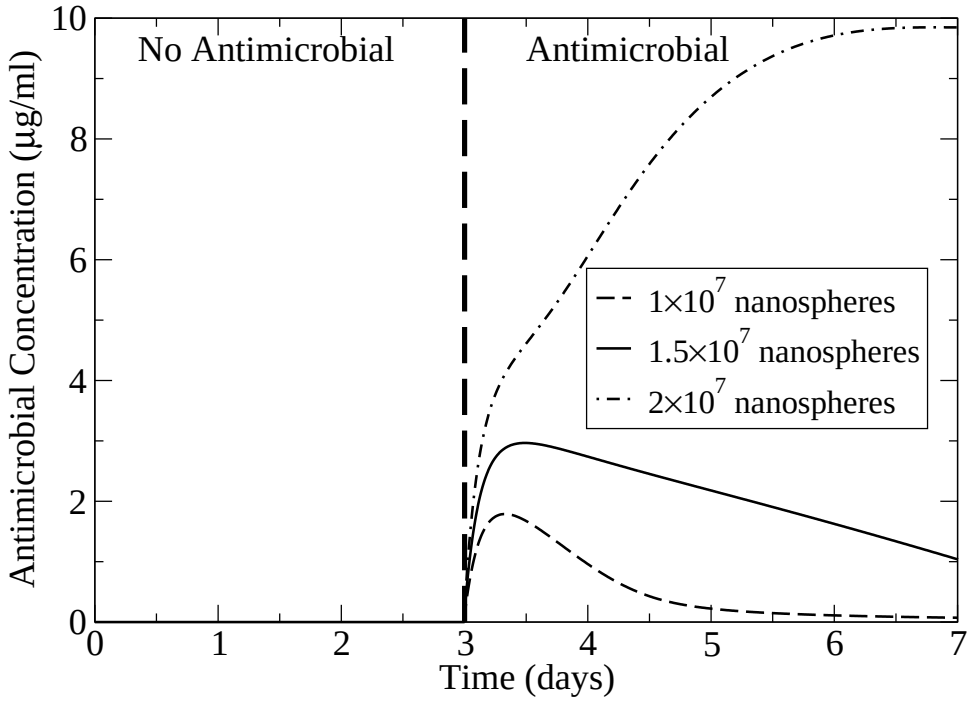

Figure 4.12: Antimicrobial concentration in biofilm where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with low initial quantity of persister bacteria

ing treatment option [23, 31]. The nanosphere fully degrades by day seven, at which time all the available antimicrobial is released. The portion of the graph during days $0-3$ is identical to that of figure 4.1 since they both used the same solution procedure for this period of time. Note that for each of the cases presented in this section for nanosphere delivery a 'no-flux' condition has been used which does not allow any antimicrobial to leave the biofilm system. Also, it is assumed that once antimicrobial is released from the nanosphere, it diffuses into the biofilm instantaneously. For the curves seen during the antimicrobial period of $4-7$ days, three outcomes are demon-

strated. In the case of 1×10^7 nanospheres, the amount of antimicrobial in the system is insufficient to stop growth, and the living bacteria growth appears unaffected. The corresponding graph in figure 4.12 showing the amount of antimicrobial in the system at various times explains that for the case of 1×10^7 nanospheres, the concentration of antimicrobial never exceeds 2μg/ml. The constant concentration study with the same initial conditions (figure 4.1) demonstrated that 4μg/ml was insufficient to immediately stop growth, and thus the dosage level that never exceeds a mere 2μg/ml with nanosphere delivery accordingly does not affect the living bacteria growth.

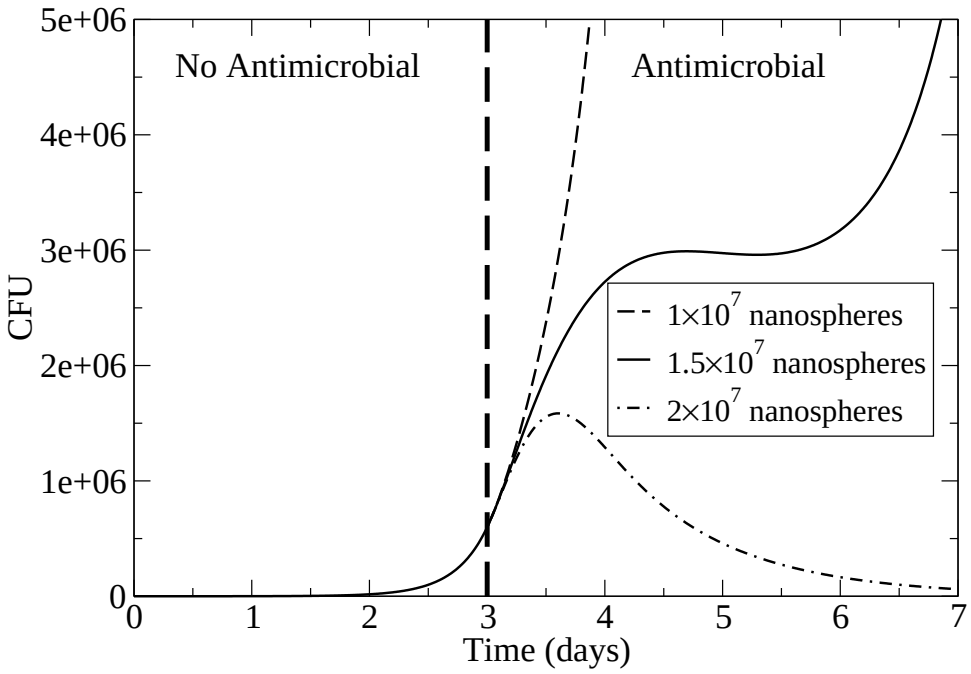

Figure 4.13: Persister bacteria growth and decay in biofilm conditions where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with low initial quantity of persister bacteria

In figure 4.11 the curve correlating to 1.5×10^7 nanospheres shows a unique growth pattern similar to that seen in [21, 22]. The living bacteria population increases approximately from day 3 to 3.75, decreases from day 3.75 until 4.75, then steadily increases thereafter. There is a two-fold explanation for this. First, the antimicrobial concentration is not constant as shown in figure 4.12. For the period of time of living bacteria population decrease (days $3.75 - 4.75$), the corresponding antimicrobial concentration is between approximately 2.3μg/ml and 3μg/ml. This concentration is sufficient to slow the growth of the living bacteria. Nonetheless, the antimicrobial concentration continues to decrease after day 4.75. As a result, the concentration of antimicrobial becomes too low to hinder the growth of living bacteria and the living bacteria population begins to grow again. For the second part of the explanation for the living bacteria curve correlating to 1.5×10^7 nanospheres, consider the graph depicting persister population growth in figure 4.13 correlating to the same number of nanospheres. During the period of time for living bacteria population decrease for days $3.75 - 4.75$, the persister population increases. After day 4.75 the persister population decreases until day 5.5, then steadily increases. This result shows that while the bacteria in the biofilm were being threatened by antimicrobial, the ability for the living bacteria to form persister bacteria acted as a mechanism of resistance. Though the population of living bacteria decreases slightly from days $3.75 - 4.75$ as a consequence of the formation of persisters, it is not detrimental to the overall viability of the biofilm. After the antimicrobial concentration decreases due to the limited amount of antimicrobial in the nanospheres, the persister population

is able to revert to living bacteria and repopulate the biofilm. For a treatment option to be effective, not only must a certain concentration of antimicrobial be present in the biofilm, but it must also be present for long enough to eradicate the living bacteria [35]. This result further demonstrates the difficulty of successfully eradicating infections caused by biofilms.

Finally, the curve correlating to 2×10^7 nanospheres on figure 4.11 shows a brief increase in the living bacteria until day 3.5, then a decrease to approximately zero living bacteria by day seven. The corresponding antimicrobial curve in figure 4.12 shows that by day 3.5 the concentration of antimicrobial has reached 4μg/ml, and increases steadily therafter until leveling off just below 10μg/ml. As shown in the constant concentration study (figure 4.1), the living bacteria population will decrease with only 4μg/ml of antimicrobial given enough time. Thus, in the case of nanosphere delivery wherein the antimicrobial concentration exceeds 4μg/ml, the living bacteria population is able to be decreased.

The height of the biofilm for the case of a low initial persister population and nanosphere delivery of antimicrobial (figure 4.14) shows that for both 1×10^7 and 1.5×10^7 nanospheres, the growth of biofilm height is unrestricted. Recall that due to the lack of any detachment terms for the calculation of the height of the biofilm, if the bacteria populations are not diminished, the biofilm height will grow without bound. With 2×10^7 nanospheres, the height of the biofilm is restricted and levels off to a slightly higher than realistic 500μm. Looking at figure 4.12, one can see the effect of the height of the biofilm on antimicrobial concentrations. In the case of

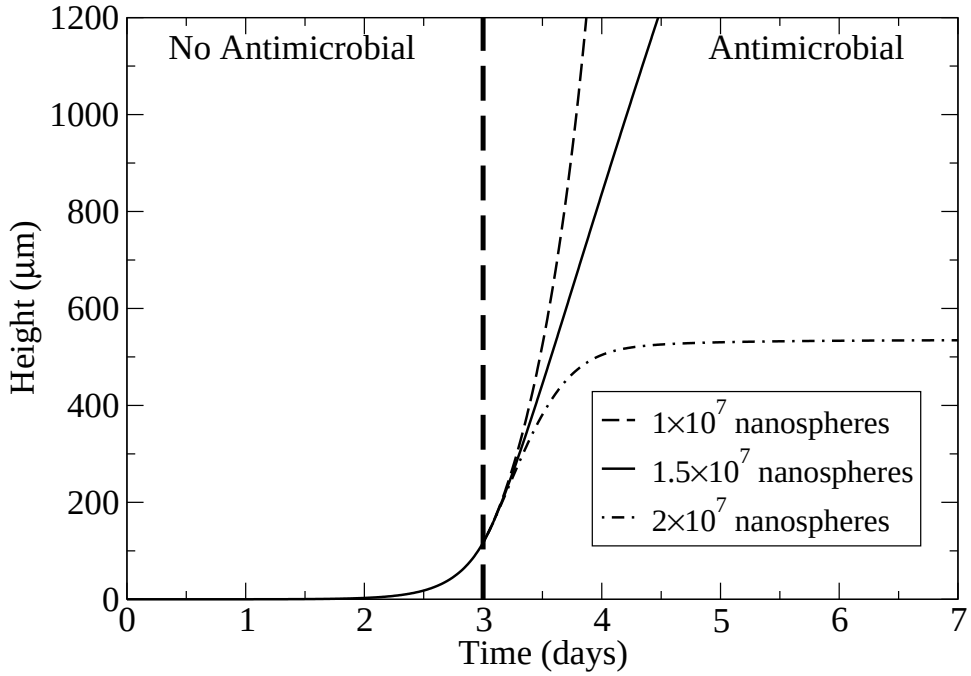

Figure 4.14: Height of biofilm where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with low initial quantity of persister bacteria

2×10^7 nanospheres where the height of the biofilm levels off, the concentration of antimicrobial also levels off. Given the no-flux condition, if the volume (equivalent to height in this 1-D model) is held constant, the concentration of antimicrobial will also remain constant. In the cases where the height of the biofilm (and thus the volume of biofilm) continued to increase (with 1×10^7, and 1.5×10^7 nanospheres) the antimicrobial concentration consequently decreases with time. As seen in equation (3.36) describing the concentration of antimicrobial in time, for a given mass of antimicrobial, as the biofilm height d increases the concentration of the antimicrobial

must decrease. Comparing figure 3.1 to figure 4.12 the disparity in the graphs of antimicrobial concentration can be explained by the increase in the height. Both the four days depicted in figure 3.1 and the last four days of figure 4.12 show antimicrobial concentrations over the span of four days during which the nanosphere degrades with identical initial height conditions and other parameter values. The difference in the antimicrobial concentrations in these two figures is due solely to the fact that in figure 3.1, the height of the biofilm is held constant, while in figure 4.11 the height of the biofilm is realistically changing. As a result, the concentrations of antimicrobial in figure 4.11 with a given quantity of nanospheres are lower than those concentrations seen in figure 3.1 with the same quantity of nanospheres. That is, figure 4.11 shows concentrations that are low, and sometimes decreasing (as in the case of 1×10^7 and 1.5×10^7 nanospheres) since the height of the one dimensional biofilm (and thus the volume of the biofilm) is increasing. Thus, one can see that laboratory testing showing concentrations of drug released from nanospheres in optimal conditions of a non-growing biofilm similar to figure 3.1 may overestimate the actual concentrations of antimicrobial seen in a realistic growing biofilm setting as seen in figure 4.11.

4.5 High Initial Persister Population with Antimicrobial Delivered via Nanospheres

In this section, the case for high initial persister population is considered. Looking at figure 4.15 it is apparent that the same number of nanospheres discussed previously has different effects on the biofilm. This is due to the actual population sizes of living bacteria at the time of antimicrobial delivery. In the case for a low initial persister

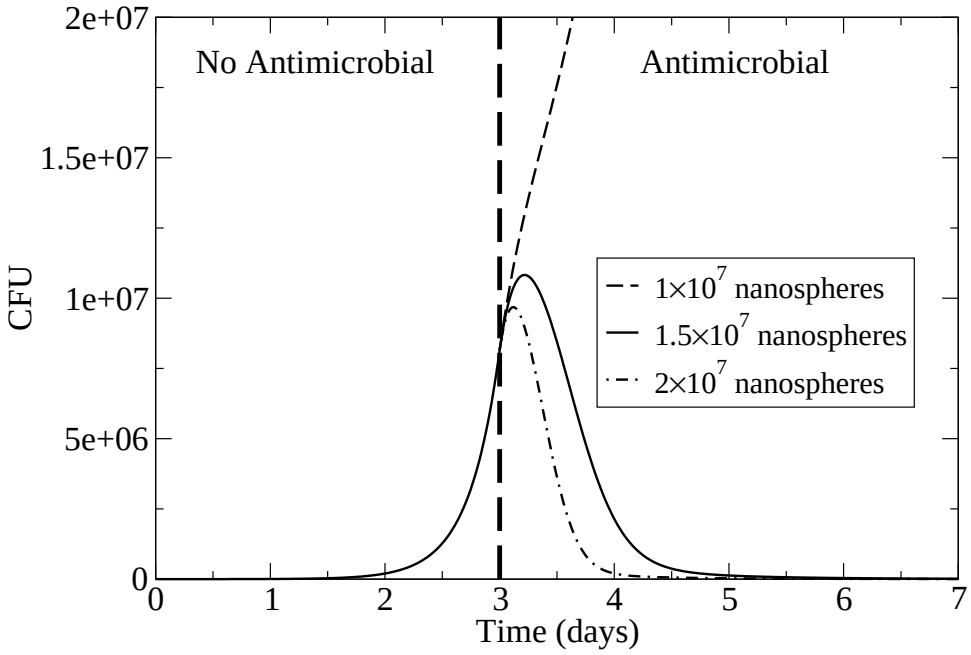

Figure 4.15: Living bacteria growth and decay in biofilm conditions where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with high initial quantity of persister bacteria

population the living bacteria population was approximately 10 million CFU at day 3. In the case of high initial persister population the living bacteria population is approximately 8 million CFU at day 3. Recall, this disparity was explained previously by the conservation term α in equation (2.30) from chapter 2 and the physical interpretation that the biofilm acclimates differently to its environment in the two cases.

As seen in figure 4.15, the nanosphere quantities of 1×10^7 and 1.5×10^7 are sufficient to decrease the living bacteria populations. Due to the population size

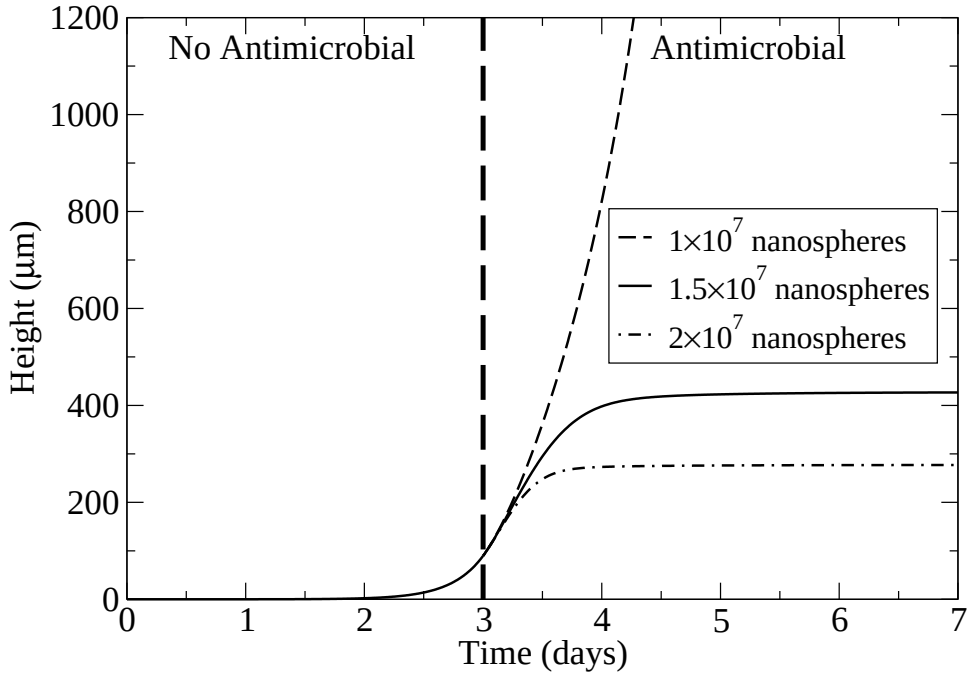

Figure 4.16: Height of biofilm where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with high initial quantity of persister bacteria

of living bacteria being smaller at day 3, the height of the biofilm is smaller at day 3 (which correlates to a smaller volume of biofilm), and thus the same number of nanospheres results in higher concentrations of antimicrobial as seen in comparing figure 4.14 to 4.16 and figure 4.12 to 4.17. The living bacteria population decreases accordingly. As a result of the higher concentrations of antimicrobial, the persister bacteria do not affect the growth of living bacteria with 1.5×10^7 nanospheres as seen previously. Consequently, the persister populations simply decrease with the living bacteria populations seen in figure 4.18 as the effect of the antimicrobial is

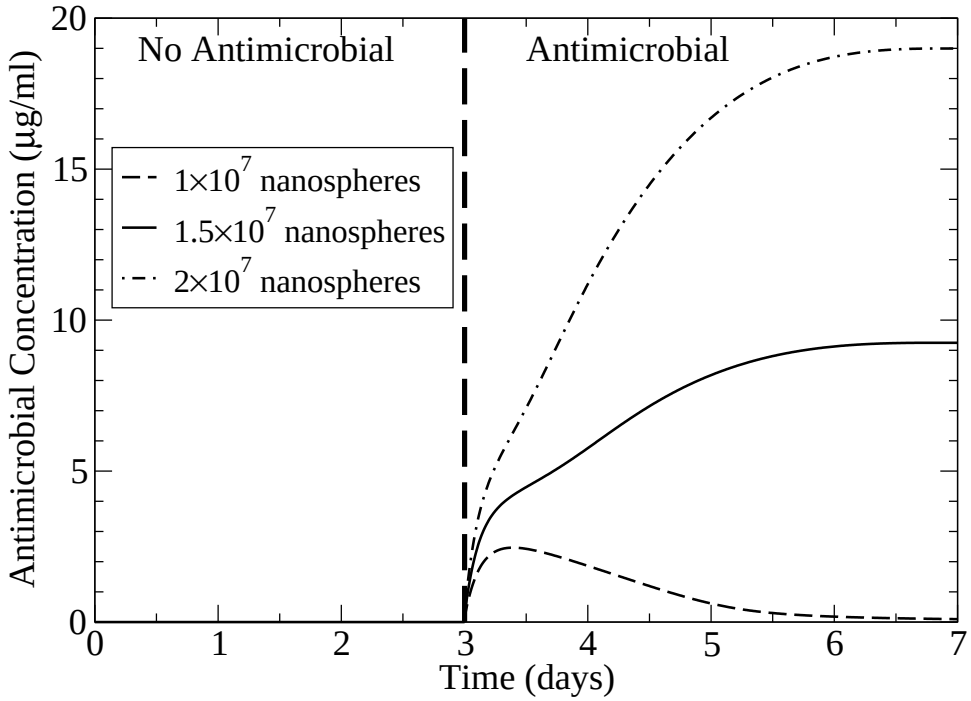

Figure 4.17: Antimicrobial concentration in biofilm where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with high initial quantity of persister bacteria

too dominant on the growth rate of living bacteria. This result suggests that given enough antimicrobial, with a small number of living bacteria, persisters may not be able to prevent the antimicrobial from eradicating an infection caused by a biofilm.

In the case of 1×10^7 nanospheres, an insufficient amount of antimicrobial is released into the biofilm to stop living bacteria growth as seen in figure 4.15. Accordingly, the persister population continues to increase seen in figure 4.18 and as a result the height of the biofilm continues to increase as seen in figure 4.16 causing the antimicrobial concentration in figure 4.17 to decrease with time.

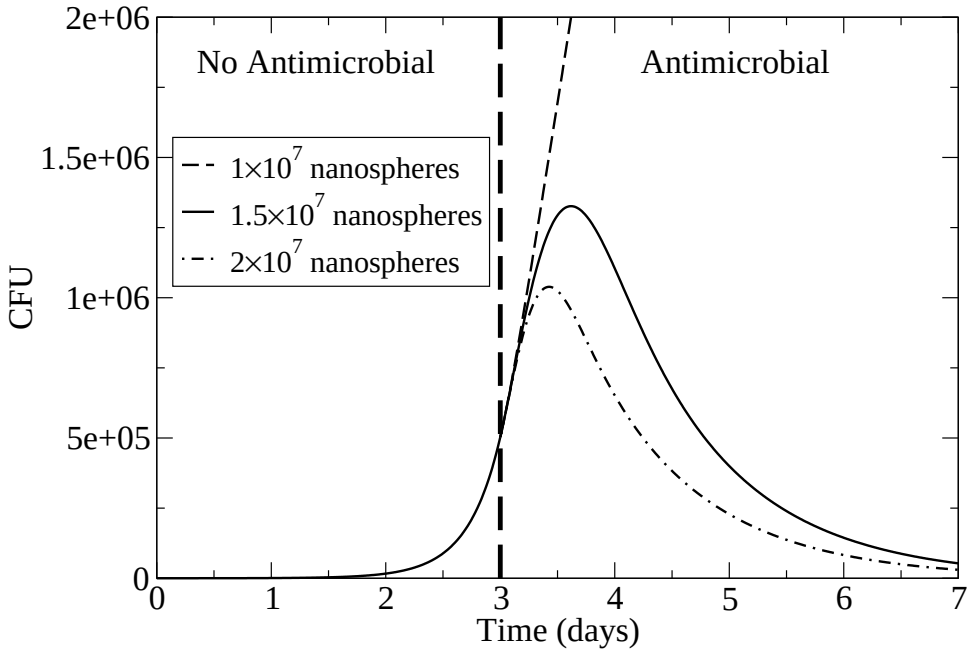

Figure 4.18: Persister bacteria growth and decay in biofilm conditions where antimicrobial is delivered by nanosphere to biofilm surface and degrades from day three until day seven with high initial quantity of persister bacteria

4.6 Demonstration of Model Utility

In this section, the manner in which this model could be applied to providing aid to medical scientists will be explained. It is the hope of any model that information beneficial to society can be ascertained. Here, a simple approximation for determining required dosage amounts of nanosphere delivered medication will be explained for human patients suffering from biofilm infections in the pulmonary region in order to see whether the model predictions are reasonable. The quantities used for calculation

have been summarized in the spreadsheet found in appendix A. The spreadsheet is composed by utilizing the calculations that follow.

Consider the scenario of in vivo drug studies being performed on mice to determine effective dosage levels to eradicate biofilm infections in the respiratory region. In these studies, a certain mass of nanoparticles, call it m (in g), is deposited into a chamber of approximate volume $V_{chamber}$ cm^3 where the mice are located. Each nanoparticle has approximate radius of r_{nano} cm and density of ρ_{nano} g/cm^3. Thus for a mass m of nanoparticles, the number of nanospheres, call it η_{nano} is defined as:

$$\eta_{nano} = \frac{m}{\rho_{nano}\frac{4}{3}\pi {r_{nano}}^3}. \tag{4.2}$$

Thus the mass m of nanospheres corresponds to a chamber concentration, $C_{chamber}$, of

$$C_{chamber} = \frac{\eta_{nano}}{V_{chamber}} \tag{4.3}$$

nanoparticles per cm^3. Each nanoparticle is loaded with antimicrobial at some percentage by weight, P_w, determined by the scientist and degrades in one day by releasing some percentage of the whole, P_r, (written as some decimal fraction of 1) which can be determined from the concentration of antimicrobial initially in the nanosphere, C_0, and the rate constant k found in table A.4 in appendix A. To determine P_r the following calculation is performed:

$$P_r = 1 - \left[1 - \frac{k(1 * 24 * 3600)}{r_{nano}C_0}\right]^3. \tag{4.4}$$

Thus each nanoparticle releases in one day a mass of antimicrobial, m_r, defined as

$$m_r = \rho_{nano}\frac{4}{3}\pi {r_{nano}}^3 P_w P_r \tag{4.5}$$

grams. The MIC of an antimicrobial with constant concentration delivered to the biofilm can be determined using the model from figure 4.1. Thus if the MIC of a drug, C_{MIC}, has been determined for a given bacterial strain in units of g/cm^3 the total number of nanoparticles, η_k, needed to kill the bacteria within the biofilm of height H_b (with assumed unitary width and length) is,

$$\eta_k = \frac{C_{MIC} * 1 * 1 * H_b}{m_r}. \tag{4.6}$$

Note that η_k is the best case scenario for the number of nanospheres what would be required to kill the biofilm. This is an underestimate of how many would be needed in reality, because this estimate assumes a constant height of the biofilm and that the amount of antimicrobial released in one day occurs instantaneously at the time of introduction of the nanospheres.

On the other hand, using the model, the number of nanospheres required to kill bacteria, η_{kmodel} in the biofilm (of surface area 1×1 cm^2) can be determined from a figure similar to figure 4.11. This value for the number of nanospheres required to kill the biofilm is more realistic as it assumes the nanosphere requires some time to degrade and thus does not instantaneously release all of the antimicrobial that is released in one day's time. Moreover, the height of the biofilm is considered non-constant for η_{kmodel}, which would increase the number of nanospheres needed to kill

the bacteria within the biofilm (since the increasing height for the one-dimensional model would imply increased biofilm volume).

Now, the recipient of the drug (deemed 'the patient') has some average respiratory volume, V_r cm^3/min. The patient also has some percentage of respired air volume that reaches the pulmonary region, P_b. Moreover, the surface area of the patient's pulmonary region can be given by A_p cm^2. Let the dosage time for breathing in nebulized nanoparticles be given by t_{dose} minutes. The value η_k determined from equation (4.6) or η_{kmodel} could be used in the following calculation. Using either of these numbers, the amount of nanoparticles that should be breathed in by the patient during nebulization, $\eta_{breathed}$ to cover the surface area of the pulmonary region can be calculated as

$$\eta_{breathed} = \frac{\eta_{kmodel} A_p}{P_b * 1 * 1}, \tag{4.7}$$

where the surface area of the modelled 1 cm $\times$ 1 cm biofilm is explicitly written in the denominator to elucidate unit analysis. Based on the dosage time, and respiratory volume, the total concentration of nanospheres that should be administered to the patient can be calculated by

$$C_{administered} = \frac{\eta_{breathed}}{V_r t_{dose}}. \tag{4.8}$$

nanospheres/cm^3. Thus the modeller can inform the clinician on proper dosage levels using the model. Moreover, the number of nanospheres needed in the nebulization

chamber to achieve this concentration level is:

$$\eta_{chamber} = C_{administered} * V_{chamber}. \tag{4.9}$$

Then, using equation (4.2) and setting $\eta_{nano} = \eta_{chamber}$ one could find the exact mass of nanospheres needed in the chamber. That is, the mass of nanospheres required in the chamber, $m_{chamber}$ is:

$$m_{chamber} = \eta_{chamber}\rho_{nano}\frac{4}{3}\pi {r_{nano}}^3. \tag{4.10}$$

In this way, effective dosage strategies can be found and the time required for drug-testing can be expedited. As demonstration of practical use, the spreadsheet found in appendix A shows values used for an in vivo study on mice. Using the MIC of 6 μg/ml in the best case scenario the number of nanospheres required to kill the biofilm is estimated as approximately 4 million. As shown in figure 4.11, the number of nanospheres actually required is much more (approximately 20 million nanospheres). Again, the disparity can be explained since the model accounts for a slow degradation of the nanosphere over the first day after application and hence slow release of antimicrobial as seen in figure 4.12 whereas the spreadsheet calculations assume an instantaneous release of antimicrobial into the biofilm at the beginning of the first day. As further explanation of the disparity, the height of the biofilm continues to increase (though slightly) for the modelled case with 20 million nanospheres as seen in figure 4.14 and thus the effective antimicrobial concentration decreases, whereas

the spreadsheet calculations assume a constant height for the biofilm. Nonetheless, calculations similar to those found in the spreadsheet give reasonable results considering the ease with which the calculations can be implemented. Using both the model described in this work and the calculations outlined in the spreadsheet together can provide valuable insight to the medical scientists performing drug-tests.

In summary, four estimates for antimicrobial interaction with biofilms are discussed in this work (all estimates assume no loss of antimicrobial). The first estimate is constant antimicrobial being delivered with fast diffusion of antimicrobial through the biofilm. In this least conservative estimate, the concentration of antimicrobial is held constant, and antimicrobial is assumed to quickly diffuse through the biofilm so that all bacteria within the biofilm are exposed to it. As a result, this estimate shows the quickest living bacteria death rates as seen in figure 4.1. The next least conservative estimate for needed antimicrobial, seen in the spreadsheet calculation, assumes all of the antimicrobial that is released from the nanospheres in one day has been released instantaneously at the start of day one. This is nearly identical to the constant concentration estimate, as all of the antimicrobial is assumed present immediately and slow release is not considered. The next least conservative estimate is the case of nanosphere delivery with fast diffusion. In this estimate, as seen in figure 4.11, living bacteria are not killed as readily as with constant antimicrobial estimates or those estimates seen by the spreadsheet calculation. This is a result of the slow release of antimicrobial resulting from the degradation of the nanosphere seen in figure 3.1 or figure 4.12, and thus limited exposure to higher antimicrobial concentrations for

bacteria in the biofilm. Finally, the most conservative estimate considered here, no diffusion of antimicrobial after release from the nanosphere, was discussed briefly in equation (3.33); since the antimicrobial is not able to diffuse through the biofilm, little effect on living bacteria populations is expected. All four aforementioned estimates consider no-flux boundary conditions and thus an estimate considering only some flux (some leakage of antimicrobial) or complete flux (complete leakage of antimicrobial) from the biofilm boundary would be the most conservative estimate. Though they are not presented in this work, one would anticipate that with leaky boundary conditions a higher amount of antimicrobial would need to be administered, as the leaky boundary conditions would limit exposure time to high concentrations of antimicrobial. Comparing these estimates enables the modeller to better understand the biofilm's interaction with antimicrobial. That is, the antimicrobial required for the least conservative estimates (constant concentration estimates and spreadsheet calculations) can provide a lower bound for what exposure to antimicrobial is needed in reality, whereas most conservative estimates (nanosphere delivery methods) can provide an upper bound for exposure to antimicrobial needed in reality. The medical scientist can use the results from this model to direct their drug-tests appropriately, saving valuable time and resources.

4.7 Conclusion

In conclusion, the persister bacteria appear to be beneficial to biofilm survival in certain conditions and could act as a mechanism of resistance. The bacteria's lack

of 'knowledge' (quorum-sensing) about the presence of antimicrobial limits the utility of persisters in the cases for high concentrations of antimicrobial. Thus, future models may want to incorporate an ability for bacteria to recognize the presence of antimicrobial as in [12] and accordingly increase the value of k_f and decrease the value of k_R to model increased resistance of biofilms to antimicrobials. Given a high enough concentration, antimicrobial applied topically to a biofilm proves to be an effective treatment option. Also, with the conditions assumed, nanosphere delivery of antimicrobial is effective. However, as the nanosphere results only consider the cases for infinite diffusion of antimicrobial into the biofilm, and a no-flux condition within the biofilm, future models should relax these restrictions to achieve more realistic results. In addition, future models may want to consider combination therapies that have been shown to be the most effective [35]. The height of the biofilm should not exceed approximately 400μm, and in some cases much smaller heights, and thus future models should include a detachment term in the biofilm height equation, and bacteria population equations to keep the height at reasonable levels. Ultimately, a two-dimensional model including detachment promoting agents and inhomogeneities in the direction of more than the vertical axis should be considered as outlined in chapter 2.

The 1-D model presented here fulfills the goal of the modeller to balance the need for accuracy of the processes explained and the desire to make the model computationally efficient. As shown, the results explain many of the phenomena witnessed in biofilm mechanics and bacterial growth including increased resistance

to antimicrobial, acclimation to the environment leading to increased biofilm growth, and conservation of resources in the biofilm (in this case modelled by conservation of volume)[1, 6]. At the same time, all calculations for a given set of initial conditions for a total growth period of 17 days (total growth period not shown in the graphs) took less than seven minutes on a standard laptop computer with 4GB of memory and an AMD Turion(tm)X2 Ultra Dual-Core Mobile ZM-80 processor. As previously stated, the calculations were all performed using MAPLE 10.

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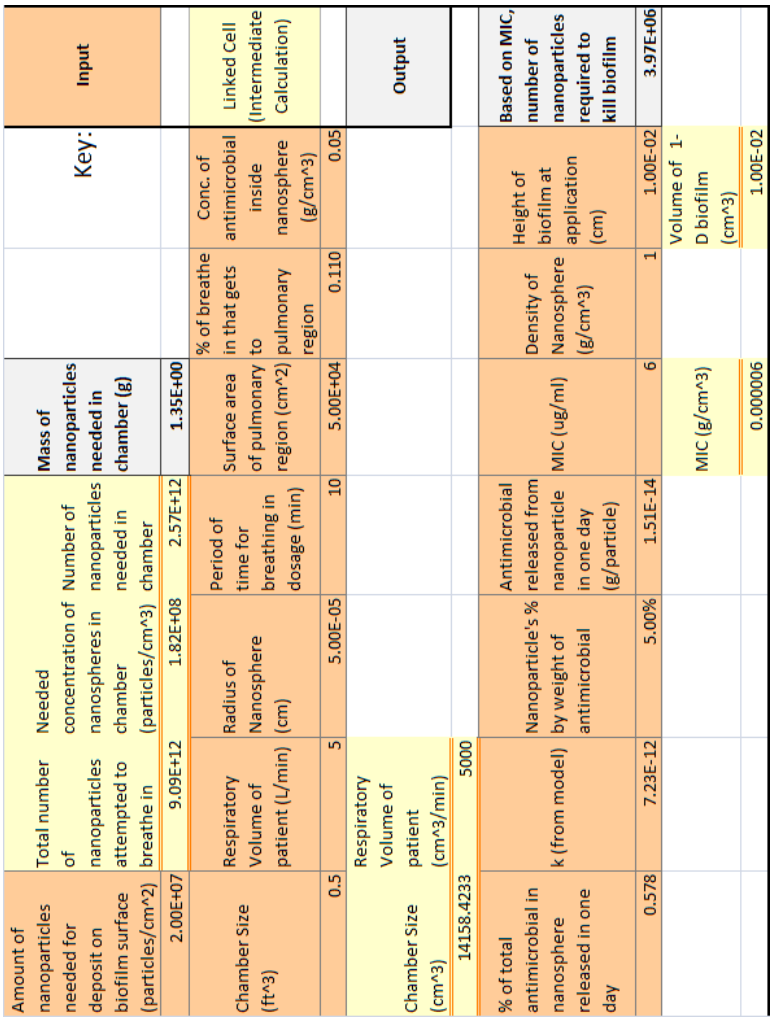
APPENDIX

VALUES USED IN SOLUTION PROCEDURE AND DISCUSSED IN RESULTS



Input						Key:			
Amount of nanoparticles needed for deposit on biofilm surface (particles/cm^2)	Total number of nanoparticles attempted to breathe in	Needed concentration of nanospheres in chamber (particles/cm^3)	Number of nanoparticles needed in chamber	Mass of nanoparticles needed in chamber (g)					Linked Cell (Intermediate Calculation)
2.00E+07	9.09E+12	1.82E+08	2.57E+12	1.35E+00					
Chamber Size (ft^3)	Respiratory Volume of patient (L/min)	Radius of Nanosphere (cm)	Period of time for breathing in dosage (min)	Surface area of pulmonary region (cm^2)	% of breathe in that gets to pulmonary region		Conc. of antimicrobial inside nanosphere (g/cm^3)		
0.5	5	5.00E-05	10	5.00E+04	0.110		0.05		
Chamber Size (cm^3)	Respiratory Volume of patient (cm^3/min)								Output
14158.4233	5000								
% of total antimicrobial in nanosphere released in one day	k (from model)	Nanoparticle's % by weight of antimicrobial	Antimicrobial released from nanoparticle in one day (g/particle)	MIC (ug/ml)	Density of Nanosphere (g/cm^3)	Height of biofilm at application (cm)			Based on MIC, number of nanoparticles required to kill biofilm
0.578	7.23E-12	5.00%	1.51E-14	6	1	1.00E-02			3.97E+06
				MIC (g/cm^3)		Volume of 1-D biofilm (cm^3)			
				0.000006		1.00E-02			

Figure A.1: Spreadsheet used for demonstrating model utility

Table A.1: Constants for part one of solution: no antimicrobial is present for low initial persister population

Constant	Description	Value	Units	Source
b	natural death rate of bacteria	1×10^{-12}	s^{-1}	estimated
B_{i0}	initial concentration of inert bacteria	0	$\frac{g}{cm^3}$	estimated
$B_i{}^*$	mass of single dead bacterium	1×10^{-12}	g	[37]
B_0	initial concentration of living bacteria	8.3333×10^{-4}	$\frac{g}{cm^3}$	derived
B_{p0}	initial concentration of persister bacteria	1.6667×10^{-4}	$\frac{g}{cm^3}$	derived
$B_p{}^*$	mass of single persister bacterium	1×10^{-12}	g	[37]
B^*	mass of single living bacterium	1×10^{-12}	g	[37]
C_{Source}	concentration of antimicrobial at biofilm surface	0	$\frac{g}{cm^3}$	assumed
C_0	concentration of antimicrobial inside nanosphere	0	$\frac{g}{cm^3}$	assumed
$\bar{D}_C$	diffusivity coefficient of the antimicrobial	1×10^{-5}	$\frac{cm^2}{s}$	[9]
Δt	time step for height iteration	600	s	estimated
$\bar{D}_S$	diffusivity coefficient of the nutrient	4×10^{-5}	$\frac{cm^2}{s}$	[17]
E_0	initial concentration of EPS	0	$\frac{g}{cm^3}$	estimated
h_0	initial height of biofilm	1.92×10^{-7}	cm	derived
k	rate at which nanosphere degrades	7.23×10^{-12}	$\frac{g}{cm^2 \cdot sec}$	derived
$k3_C$	coefficient of delta function in antimicrobial equation	0	none	assumed
k_C	rate at which antimicrobial is lost	0	s^{-1}	assumed
k_f	rate at which living bacteria change into persister bacteria	5.83333×10^{-7}	s^{-1}	[11]
k_{HC}	mass transfer coefficient through the top of the biofilm	1×10^{-4}	$\frac{cm}{s}$	derived
k_{HS}	diffusivity of nutrient through the top of the biofilm	1×10^{-3}	$\frac{cm}{s}$	derived
k_R	rate at which persister bacteria change into living bacteria	1.17×10^{-5}	s^{-1}	estimated
K_S	saturation level of nutrient	1×10^{-4}	$\frac{g}{cm^3}$	estimated
k_S	rate at which nutrient is lost from biofilm	2.5×10^{-4}	s^{-1}	[11, 17]
k_{SC}	mass transfer coefficient through the bottom of the biofilm	0	$\frac{cm^2}{s}$	assumed
κ	efficiency with which antimicrobial is converted into bacterial death	0	none	assumed
κ_{EPS}	efficiency with which bacteria create EPS	.0033	none	derived from [12]
κ_g	efficiency with which bacteria convert nutrient into growth	6.3	none	estimated
μ_S	rate of nutrient transfer into bacteria	8.33×10^{-5}	s^{-1}	[17]
ϕ	porosity of biofilm	0.995	none	estimated
R_C	radius of the nanosphere containing antimicrobial	1×10^{-5}	cm	none
ρ_B	density of the living bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_i}	density of the inert bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_p}	density of the persister bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_E	density of the extracellular polymeric substance (EPS)	0.033	$\frac{g}{cm^3}$	[13]
S_{Source}	concentration of nutrient at biofilm surface	1×10^{-5}	$\frac{g}{cm^3}$	estimated
t_{final}	total time of biofilm growth	17	$days$	none
t_{med}	time that antimicrobial is applied	259200	s	none
Y_S	effectiveness of antimicrobial killing bacteria	0.8	$\frac{cm^3}{g}$	[9]
z_0	nanosphere location along vertical axis	5×10^{-4}	cm	estimated

Table A.2: Constants for part one of solution: no antimicrobial is present for high initial persister population

Constant	Description	Value	Units	Source
b	natural death rate of bacteria	1×10^{-12}	s^{-1}	estimated
B_{i0}	initial concentration of inert bacteria	0	$\frac{g}{cm^3}$	estimated
$B_i{}^*$	mass of single dead bacterium	1×10^{-12}	g	[37]
B_0	initial concentration of living bacteria	$(160/2.24) \times 10^{-5}$	$\frac{g}{cm^3}$	derived
B_{p0}	initial concentration of persister bacteria	$(64/2.24) \times 10^{-5}$	$\frac{g}{cm^3}$	derived
$B_p{}^*$	mass of single persister bacterium	1×10^{-12}	g	[37]
B^*	mass of single living bacterium	1×10^{-12}	g	[37]
C_{Source}	concentration of antimicrobial at biofilm surface	0	$\frac{g}{cm^3}$	assumed
C_0	concentration of antimicrobial inside nanosphere	0	$\frac{g}{cm^3}$	assumed
$\bar{D}_C$	diffusivity coefficient of the antimicrobial	1×10^{-5}	$\frac{cm^2}{s}$	[9]
Δt	time step for height iteration	600	s	estimated
$\bar{D}_S$	diffusivity coefficient of the nutrient	4×10^{-5}	$\frac{cm^2}{s}$	[17]
E_0	initial concentration of EPS	0	$\frac{g}{cm^3}$	estimated
h_0	initial height of biofilm	1.92×10^{-7}	cm	derived
k	rate at which nanosphere degrades	7.23×10^{-12}	$\frac{g}{cm^2 \cdot sec}$	derived
$k3_C$	coefficient of delta function in antimicrobial equation	0	none	assumed
k_C	rate at which antimicrobial is lost	0	s^{-1}	assumed
k_f	rate at which living bacteria change into persister bacteria	5.83333×10^{-7}	s^{-1}	[11]
k_{HC}	mass transfer coefficient through the top of the biofilm	1×10^{-4}	$\frac{cm}{s}$	derived
k_{HS}	diffusivity of nutrient through the top of the biofilm	1×10^{-3}	$\frac{cm}{s}$	derived
k_R	rate at which persister bacteria change into living bacteria	1.17×10^{-5}	s^{-1}	estimated
K_S	saturation level of nutrient	1×10^{-4}	$\frac{g}{cm^3}$	estimated
k_S	rate at which nutrient is lost from biofilm	2.5×10^{-4}	s^{-1}	[11, 17]
k_{SC}	mass transfer coefficient through the bottom of the biofilm	0	$\frac{cm^2}{s}$	assumed
κ	efficiency with which antimicrobial is converted into bacterial death	0	none	assumed
κ_{EPS}	efficiency with which bacteria create EPS	.0033	none	derived from [12]
κ_g	efficiency with which bacteria convert nutrient into growth	6.3	none	estimated
μ_S	rate of nutrient transfer into bacteria	8.33×10^{-5}	s^{-1}	[17]
ϕ	porosity of biofilm	0.995	none	estimated
R_C	radius of the nanosphere containing antimicrobial	1×10^{-5}	cm	none
ρ_B	density of the living bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_i}	density of the inert bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_p}	density of the persister bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_E	density of the extracellular polymeric substance (EPS)	0.033	$\frac{g}{cm^3}$	[13]
S_{Source}	concentration of nutrient at biofilm surface	1×10^{-5}	$\frac{g}{cm^3}$	estimated
t_{final}	total time of biofilm growth	17	$days$	none
t_{med}	time that antimicrobial is applied	259200	s	none
Y_S	effectiveness of antimicrobial killing bacteria	0.8	$\frac{cm^3}{g}$	[9]
z_0	nanosphere location along vertical axis	5×10^{-4}	cm	estimated

Table A.3: Constants for part two of solution: constant concentration of antimicrobial applied to biofilm surface for low and high initial persister populations

Constant	Description	Value	Units	Source
b	natural death rate of bacteria	1×10^{-12}	s^{-1}	estimated
B_{i0}	initial concentration of inert bacteria	$B_{i_{final}}$ from part 1	$\frac{g}{cm^3}$	none
$B_i{}^*$	mass of single dead bacterium	1×10^{-12}	g	[37]
B_0	initial concentration of living bacteria	B_{final} from part 1	$\frac{g}{cm^3}$	none
B_{p0}	initial concentration of persister bacteria	$B_{p_{final}}$ from part 1	$\frac{g}{cm^3}$	none
$B_p{}^*$	mass of single persister bacterium	1×10^{-12}	g	[37]
B^*	mass of single living bacterium	1×10^{-12}	g	[37]
C_{Source}	concentration of antimicrobial at biofilm surface	variable	$\frac{g}{cm^3}$	assumed
C_0	concentration of antimicrobial inside nanosphere	0	$\frac{g}{cm^3}$	assumed
$\bar{D}_C$	diffusivity coefficient of the antimicrobial	1×10^{-5}	$\frac{cm^2}{s}$	[9]
Δt	time step for height iteration	600	s	estimated
$\bar{D}_S$	diffusivity coefficient of the nutrient	4×10^{-5}	$\frac{cm^2}{s}$	[17]
E_0	initial concentration of EPS	E_{final} from part 1	$\frac{g}{cm^3}$	none
h_0	initial height of biofilm	h_{final} from part 1	cm	none
k	rate at which nanosphere degrades	7.23×10^{-12}	$\frac{g}{cm^2 \cdot sec}$	derived
$k3_C$	coefficient of delta function in antimicrobial equation	0	none	assumed
k_C	rate at which antimicrobial is lost	0	s^{-1}	assumed
k_f	rate at which living bacteria change into persister bacteria	5.83333×10^{-7}	s^{-1}	[11]
k_{HC}	mass transfer coefficient through the top of the biofilm	1×10^{-4}	$\frac{cm}{s}$	derived
k_{HS}	diffusivity of nutrient through the top of the biofilm	1×10^{-3}	$\frac{cm}{s}$	derived
k_R	rate at which persister bacteria change into living bacteria	1.17×10^{-5}	s^{-1}	estimated
K_S	saturation level of nutrient	1×10^{-4}	$\frac{g}{cm^3}$	estimated
k_S	rate at which nutrient is lost from biofilm	2.5×10^{-4}	s^{-1}	[11, 17]
k_{SC}	mass transfer coefficient through the bottom of the biofilm	0	$\frac{cm^2}{s}$	assumed
κ	efficiency with which antimicrobial is converted into bacterial death	1×10^6	none	estimated
κ_{EPS}	efficiency with which bacteria create EPS	.0033	none	derived from [12]
κ_g	efficiency with which bacteria convert nutrient into growth	6.3	none	estimated
μ_S	rate of nutrient transfer into bacteria	8.33×10^{-5}	s^{-1}	[17]
ϕ	porosity of biofilm	0.995	none	none
R_C	radius of the nanosphere containing antimicrobial	1×10^{-5}	cm	estimated
ρ_B	density of the living bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_i}	density of the inert bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{Bp}	density of the persister bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_E	density of the extracellular polymeric substance (EPS)	0.033	$\frac{g}{cm^3}$	[13]
S_{Source}	concentration of nutrient at biofilm surface	1×10^{-5}	$\frac{g}{cm^3}$	estimated
t_{final}	total time of biofilm growth	17	$days$	assumed
t_{med}	time that antimicrobial is applied	259200	s	assumed
Y_S	effectiveness of antimicrobial killing bacteria	0.8	$\frac{cm^3}{g}$	[9]
z_0	nanosphere location along vertical axis	5×10^{-4}	cm	assumed

Table A.4: Constants for part two of solution: antimicrobial delivered through nanospheres

Constant	Description	Value	Units	Source
b	natural death rate of bacteria	1×10^{-12}	s^{-1}	estimated
B_{i0}	initial concentration of inert bacteria	$B_{i_{final}}$ from part 1	$\frac{g}{cm^3}$	none
$B_i{}^*$	mass of single dead bacterium	1×10^{-12}	g	[37]
B_0	initial concentration of living bacteria	B_{final} from part 1	$\frac{g}{cm^3}$	none
B_{p0}	initial concentration of persister bacteria	$B_{p_{final}}$ from part 1	$\frac{g}{cm^3}$	none
$B_p{}^*$	mass of single persister bacterium	1×10^{-12}	g	[37]
B^*	mass of single living bacterium	1×10^{-12}	g	[37]
C_{Source}	concentration of antimicrobial at biofilm surface	0	$\frac{g}{cm^3}$	assumed
C_0	concentration of antimicrobial inside nanosphere	0.05	$\frac{g}{cm^3}$	estimated
$\bar{D}_C$	diffusivity coefficient of the antimicrobial	1×10^{-5}	$\frac{cm^2}{s}$	[9]
Δt	time step for height iteration	600	s	estimated
$\bar{D}_S$	diffusivity coefficient of the nutrient	4×10^{-5}	$\frac{cm^2}{s}$	[17]
E_0	initial concentration of EPS	E_{final} from part 1	$\frac{g}{cm^3}$	none
h_0	initial height of biofilm	h_{final} from part 1	cm	none
k	rate at which nanosphere degrades	7.23×10^{-12}	$\frac{g}{cm^2 \cdot sec}$	derived
$k3_C$	coefficient of delta function in antimicrobial equation	0	none	assumed
k_C	rate at which antimicrobial is lost	0	s^{-1}	assumed
k_f	rate at which living bacteria change into persister bacteria	5.83333×10^{-7}	s^{-1}	[11]
k_{HC}	mass transfer coefficient through the top of the biofilm	0	$\frac{cm}{s}$	derived
k_{HS}	diffusivity of nutrient through the top of the biofilm	1×10^{-3}	$\frac{cm}{s}$	derived
k_R	rate at which persister bacteria change into living bacteria	1.17×10^{-5}	s^{-1}	estimated
K_S	saturation level of nutrient	1×10^{-4}	$\frac{g}{cm^3}$	estimated
k_S	rate at which nutrient is lost from biofilm	2.5×10^{-4}	s^{-1}	[11, 17]
k_{SC}	mass transfer coefficient through the bottom of the biofilm	0	$\frac{cm^2}{s}$	assumed
κ	efficiency with which antimicrobial is converted into bacterial death	$1 \times 10^6 \times m_{spheres}$	none	estimated
κ_{EPS}	efficiency with which bacteria create EPS	.0033	none	derived from [12]
κ_g	efficiency with which bacteria convert nutrient into growth	6.3	none	estimated
μ_S	rate of nutrient transfer into bacteria	8.33×10^{-5}	s^{-1}	[17]
ϕ	porosity of biofilm	0.995	none	none
R_C	radius of the nanosphere containing antimicrobial	5×10^{-5}	cm	estimated
ρ_B	density of the living bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{B_i}	density of the inert bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_{Bp}	density of the persister bacteria	0.2	$\frac{g}{cm^3}$	[13]
ρ_E	density of the extracellular polymeric substance (EPS)	0.033	$\frac{g}{cm^3}$	[13]
S_{Source}	concentration of nutrient at biofilm surface	1×10^{-5}	$\frac{g}{cm^3}$	estimated
t_{final}	total time of biofilm growth	17	$days$	assumed
t_{med}	time that antimicrobial is applied	259200	s	assumed
Y_S	effectiveness of antimicrobial killing bacteria	0.8	$\frac{cm^3}{g}$	[9]
z_0	nanosphere location along vertical axis	5×10^{-4}	cm	assumed